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The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

03006061.0

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

R C van Dijk

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Anmelder/Applicant(s)/Demandeur(s):

Kantonspital Basel
Hebelstrasse 30
4031 Basel
SUISSE
Universität Bern
Hochschulstrasse 4
3012 Bern
SUISSE

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(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

New radiolabeled conjugates based on substance p and the used thereof

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KBS 101

New radiolabeled conjugates based on substance P and the use thereof

The invention relates to new radiolabeled conjugates based on substance P and analogues thereof with the prochelator DOTAGA(tBu)4 (1-(1-carboxy-3-carboxytertbutoxypropyl)-4,7,10 (carbotertbutoxymethyl)-1,4,7,10-tetraazacyclododecane or prochelator DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1;4,7,-tris(acetic-acid-t-butyl-ester)-10-acetic-acid and the subsequent chelators and the use thereof for targeting and treatment of brain tumors.

Substance P is a naturally occuring 11-amino acid neuropeptide and is a powerful member of tachykinins initially isolated as crude extract from equine brain and gut by J.H. Gaddum and Von Euler as unidentified depressor substance in certain tissue extract **(J. Physiol. 72 (1931), 74-87 and J.H. Gaddum and H.O. Schild in J. Physiol. 83 (1934) 1-14.**

In 1971 Chang et al identified the amino acid structure of this undecapeptide as H-Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH_2 in bovine hypothalamus (Nat. New Biol. 232 (1971) 229-308. All further developments have been published by Selena Harrison and Pierangelo Geppetti to some extent in **International Journal of Biochemistry & Cell Biology 33 (2001) 555-576.**

Substance P belongs to the family of bioactive neuropeptides known as the tachykinins **(Payan (1989) Ann. Rev.Med. 40, 341).** It is believed to be involved in the transmission of pain sensation as well as a host of other biological functions.

In WO 98/03194 (Mark, L. Witten) it is claimed that substance P aerosols are effective in replenishing immune systems compromised by environmental toxicants. They are also useful for prophylaxis against treatment of infections and neoplasms. Furthermore they are useful for accelerating maturation of immune systems or maintaining immune function as well. Chronic exposure to hydrocarbons is particularly damaging to the immune system, and thus this to be named occupational hazard can be

counteracted by treatment with substance P aerosols. Especially it can enhance the immune response to a viral infection or to a bacterial infection. Furthermore it can prevent metastasis in melanoma, leukaemia or lymphoma or cancer of breast, lung, brain or kidney.

In **WO 93/18797 (G.J. Jacob et al)** a method of intraoperatively detecting and locating tumoral tissues in the body of a warm-blooded living being is claimed, comprising (a) parenterally administering to said living being a pharmaceutical composition comprising, in a quantity sufficient for detection a peptide compound, e.g. substance P and analogues labeled with a low-energy gamma photon emitting radionuclide, and then (b) after allowing the active substance to be taken up in the tumoral tissues and after blood clearance of radioactivity. Preferably the labeled peptide compound to be used in the described method is provided, directly or through a spacing group, with a chelating group.

Ivo M. Henning et al published in **Int.J. Cancer 61, 786 – 792 (1995)** in their study that SP receptors are also expressed in various types of tumors, including glioblastomas and astrocytomas, as well as in peritumoral vasculature associated with several types of solid tumors.
The aim of the study was to investigate a representative number of human neoplasms for the presence of SPR, using receptor autoradiography, to compare the results with the presence and localization for SP in normal human SP target tissue; and finally to compare the presence of SPR with somatostatin-receptor (SSR) status in the same neoplastic specimen. The comparison was in favor of SPR.

In **WO 92/18536** a method for detecting and localizing tissues, having neurokinine 1 receptors, such as tumors with NK1 receptors in the body of a warm-blooded living being, by administration of a composition, comprising a labeled small peptide, e.g. substance P or its Tyro derivative having a selective affinity to neurokinine 1 receptors, and by then assaying that living being is claimed. Furthermore the invention relates to a method for the therapeutic treatment of malignant tumors, e.g. glioma, small cell lung cancer etc..

In **US-P 5,736,120** a method is claimed for the preparation of a radiolabeled peptide composition by combining a protected polyaminocarboxylate ligand with a peptide in solid phase peptide synthesiser and complexing the chelate-peptide conjugate with a radiolnuclide such as lanthanides and

actinides. The radiolabeled peptides, including substance P can be used as diagnostic or therapeutic medical tools.
The treatment of tumors, especially brain tumor such as glioma has not been mentioned.

In **EP 0 835 662** a pharmaceutical composition is claimed, useful for killing or inhibiting multiplication of cancer cells, e.g.useful in preventing, inhibiting or modulating the hypersecretion of VIP, somatostatins, bombesin or substance P or analogues thereof. Further the combination of the above mentioned peptides may be used.

The active compound of the compositions are especially unlabeled analogues of somatostatin and substance P used for the treatment of tumor or cancer cells, particularly for the treatment of leukemia, lymphoma, adenocarcinoma of the stomach, pancreas or prostate or cancer of lung, breast, kidney or particularly rectum and colon, whereas the treatment of brain tumors has not been mentioned.

In **US-P 6,063,758** a conjugate is claimed comprising unlabeled substance P and analogs thereof, and saporin.
This invention embraces the above mentioned conjugate as well as a method of reducing the perception of pain by a subject comprising administering to the subject an effective amount of the pharmaceutical composition comprising substance P and analogues and saporin, so as to reduce the perception of pain by the subject.
The treatment of tumors, especially brain tumors has not been mentioned.

In **WO97/13410** are hybrid molecules claimed molecules containing a first part, a second part and third part connected by covalent bonds. The first part contains a chemical entity to be introduced into an animal cell. The second part contains a polypeptide effective to translocate the chemical entity across the cytoplamic membrane and into the cytoplasm of the animal cell. The third part contains a C-terminal amidated polypeptide ligand effective to cause the hybrid molecule to bind to the animal cell.
The amidated hybrid molecules exhibit surprisingly much greater biological activity than non-amidated counterparts. Preferred hybrid molecules are fusion toxins which contain an amidated neuropeptide, e.g. substance P or gastrin releasing peptide. Methods of preparing the hybrid molecules, which in preferred embodiments are prepared enzymatically by peptidiglycine-α-amidating monooxygenase, are also disclosed.

Additionally disclosed are methods of using the hybrid molecules for medical purposes, such as for selective delivery to target cells, particularly delivery of toxins to cancer cells. The advantage of said hybrid molecules is that they are more active than similar compounds which are not amidated. The active compounds used are different from the radiolabeled conjugates based on substance P and analogues and prochelators according to the invention and are not labeled conjugates of substance P and analogues.

Succesful studies on locoregional regulatory peptide receptor targeting with the diffusible somatostatin analogue ^{90}Y-labeled-dota^{0}-$_{D}$-phe^{1}-tyr^{3}-octreotide (DOTATOC), a radiolabeled conjugate, show for the first time in human brain tumors that DOTATOC has high affinity binding to SSR-2 in the low nanomolar range which has been reported by **A. Merlo et al (199) in Clin. Cancer Res.5, 1025-1033.**

Furthermore S. Hofer et al report in **Swiss Med Wkly 2001, 131: 640-644** the effect of diffusible brachytherapy (dBT) using the locally injected stable radioconjugate ^{90}Y-labeled-dota^{0}-$_{D}$-phe^{1}-tyr^{3}-octreotide (DOTATOC) for managing symptomatic low grade gliomas.

Referring to the above mentioned publications T. Schumacher et al report in **European Journal of Nuclear Medicine Vol. 29, No.4, April 2002** on their more thorough clinical experience with the novel compound, being a radiolabeled peptidic vector, focussing on low-grade and anaplastic gliomas.

Surprisingly it has been found that new radiolabeled conjugates according to the invention based on substance P and analogues thereof with the very special prochelator (chelator) DOTAGA(tBu)4 (1-(1-arboxy-3-carboxytert-butoxypropyl)-4,7,10 (carbotertbutoxymethyl)-1,4,7,10-tetraazacyclo-dodecane or chelator DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid are more effective than the earlier mentioned radioconjugate DOTATOC, the radiolabeled substance P, substance P and anlogues and saporin, and other small radiolabeled peptides with or without chelating agent in targeting and treatment of tumors, especially brain tumors,e.g. gliomas.

The prochelator (chelator) DOTAGA(tBu)4 (1-(1-carboxy-3-carboxytertbutoxypropyl)-4,7,10 (carbotertbutoxymethyl)-1,4,7,10-tetraazacyclododecane is described and claimed in **PCT/EP01/05483 (H.R. Mäcke et al)** and the prochelator DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-

dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid is fully described in **Chem. Eur. J. 1999, 5, No. 7** being part of the new radiolabeled conjugates according to the invention.

The before mentioned application **PCT/EP01/05483 (H.R. Mäcke et al)** relates to convenient synthesis of novel bifunctional prochelator DOTAGA(tBu)4 for coupling to bioactive petides for radiometal labeled peptides as conjugates that can be prepared while using above mentioned prochelator.

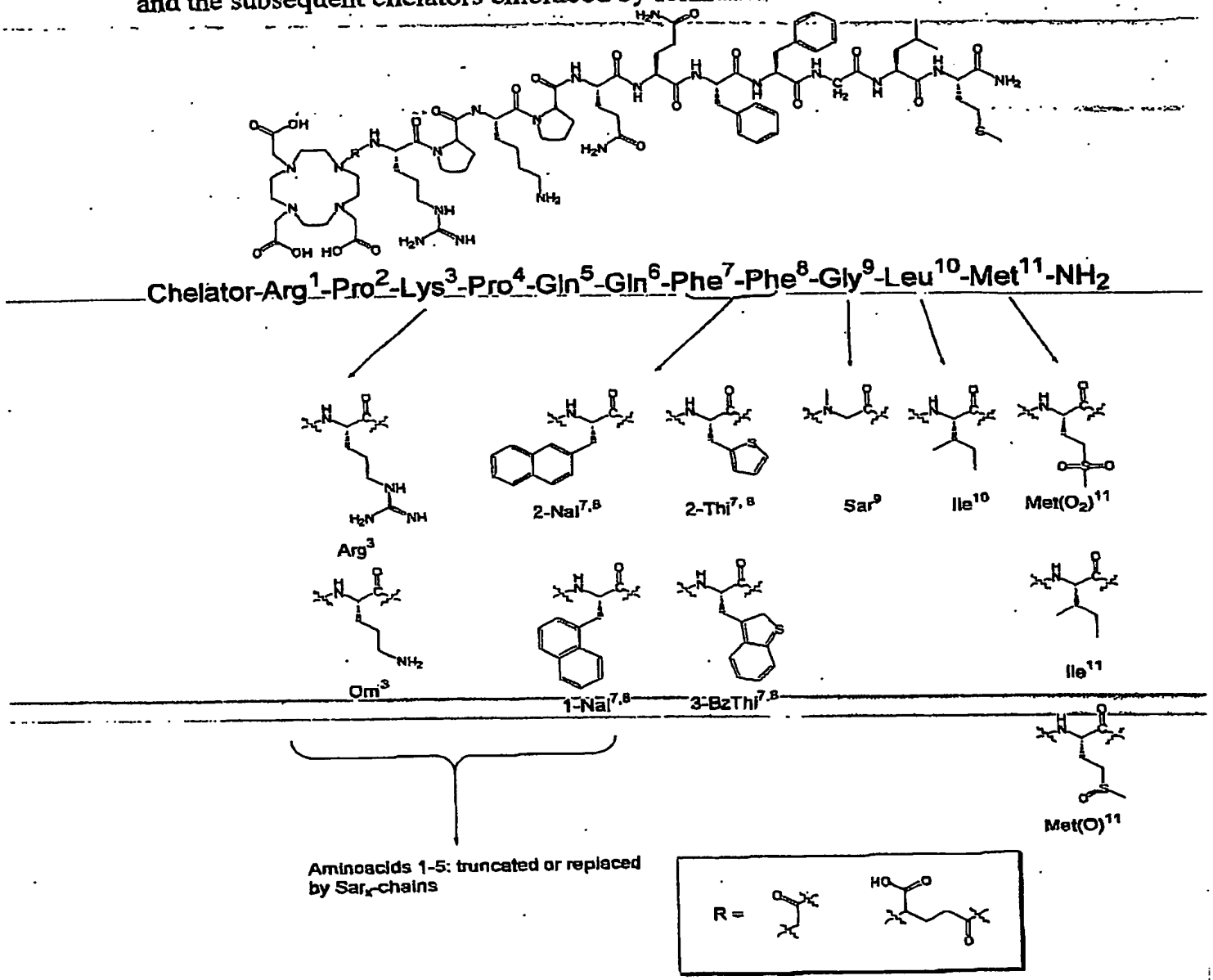
The prochelator DOTAGA(tBu)4 is prepared, e.g. by starting from a relevant amino acid and synthesizing the α-bromo-derivative thereof according to **PCT/EP01/05483 (H.R. Mäcke et al)**. This derivative is orthogonally protected (tBu, Bzl). This alkylating agent will be reacted with cyclen, cyclam etc. to form a 1:1 adduct followed by tris-alkylation with bromoacetic acid tert.-butylester and catalytic hydrogenation with for example H_2/Pd.

The synthon is monoreactive, carrying a free carboxylate for coupling to the N-terminal end of the peptide and can be coupled to any biomolecule.

The prochelator DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid is synthesized according to **Chem. Eur. J. 1999, 5, No. 7** in 3 steps starting from 1,4,7,10-tetraazacyclododecane, passing the 1,4,7,10-tetraazacyclododecane-1-carboxymethyl-benzyl ester and thereafter the 1,4,7,10-tetraazacyclododecane-4,7,10-tricarboxymethyl-tert.-butylester-1-carboxymethyl-benzylester and obtaining thereafter by hydrogenation the above mentioned prochelator DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid which is commercially available.

The invention relates to new radiolabeled conjugates based on substance P and analogues thereof with the prochelator DOTAGA(tBu)4 (1-(1-carboxy-3-carboxytertbutoxypropyl)-4,7,10 (carbotertbutoxymethyl)-1,4,7,10-tetraazacyclododecane or the prochelator DOTA (^{t}Bu)$_3$(1,4,7,10-

tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid and the subsequent chelators embraced by formula I



I

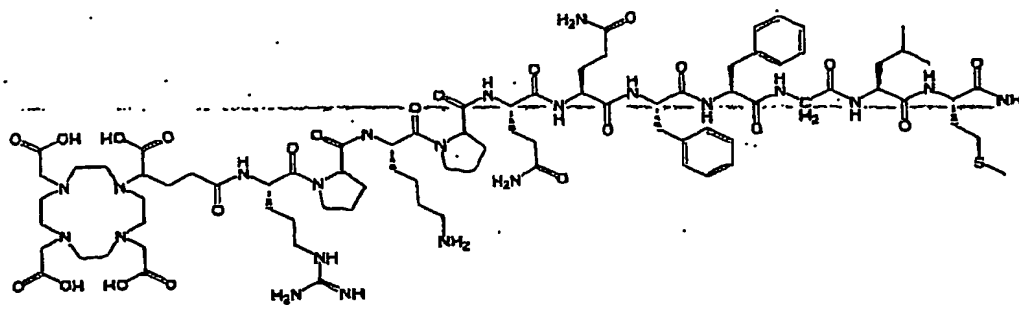
Legend: 2-Nal: 3-(2-naphthyl)-alanine; 1-Nal: 3-(1-naphthyl)-alanine; 2-Thi: 3-(2-thienyl)-alanine; Sar: sarcosine; Met(O): Methionine-sulfoxide;

Met(O_2): Methionine-sulfone; 3-BzThi: 3-(3-benzothienyl)-alanine; Orn: ornithine; Ile: isoleucine.

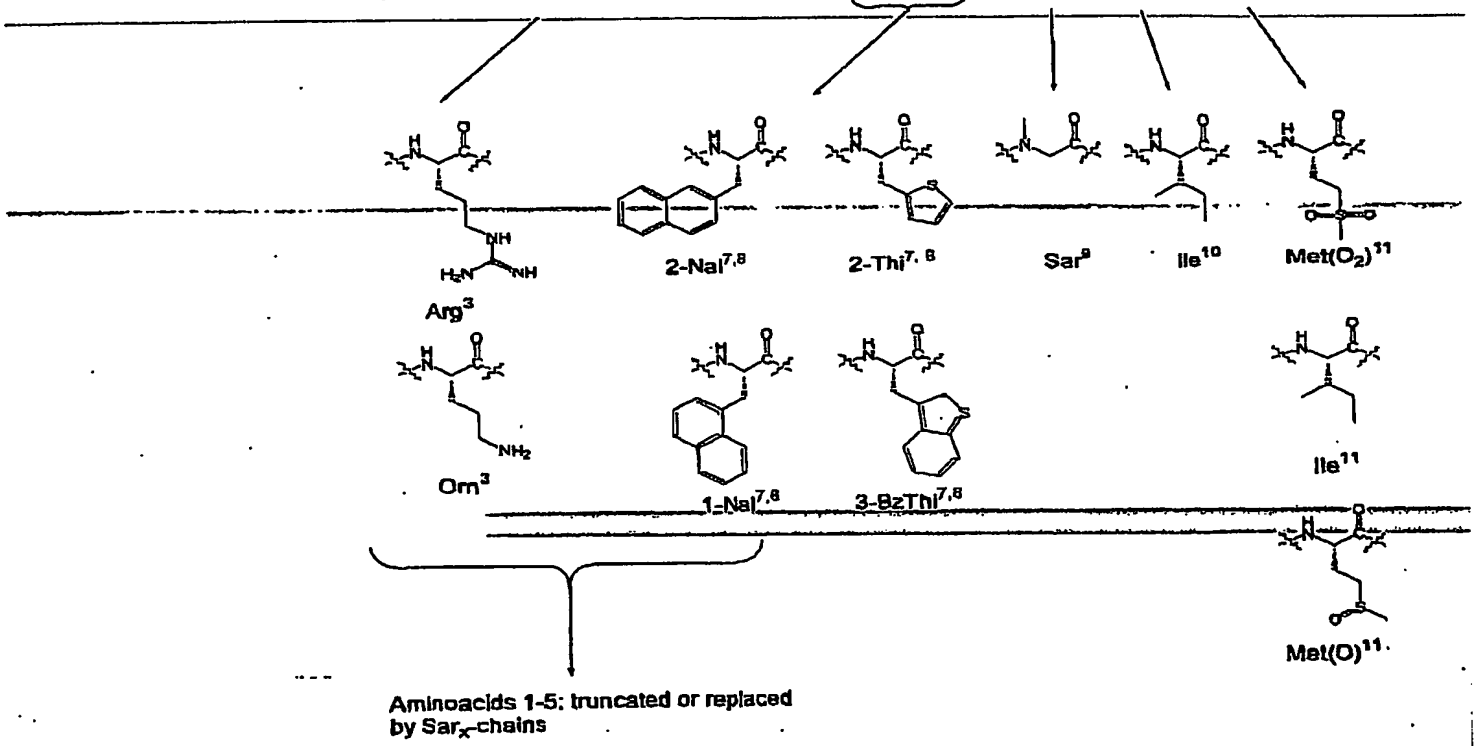
wherein the substance P and anlogue conjugates of formula I are labeled with metal isotopes selected from the group consisting of Actinium-225, Bismut-213, Cupper-64, Gallium-66, Gallium-68, Lutetium-177, Indium-111, Rhenium-186, Rhenium-188, Yttrium-86 and Yttrium-90 or the like known for radiolabeling.

Known metal isotopes for radiolabeling are for example selected from the group consisting of Tc-99m, Pb-203, Ga-68, Indium-113,Ru-97, Cu-62, Fe-52, Fe-56, Mn-52m, Mn-55, Cr-51, Cr-52, Na-23, Gd-157, Dy-162, Dy-165 As-77, Cu-67, Er-160, Sn-121, Te-127, Pr-142, Pr-143, Au-198, and Pd-109 and may be replaced by any other appropriate known isotopes.

More particularly the invention relates to new radiolabeled conjugates based on substance P and analogues thereof with the prochelator DOTAGA(tBu)4 (1-(1-carboxy-3-carboxytertbutoxypropyl)-4,7,10 (carbotertbutoxymethyl)-1,4,7,10-tetraazacyclododecane of formula Ia



DOTAGA-Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Phe^{7}-Phe^{8}-Gly^{9}-Leu^{10}-Met^{11}-NH$_2$

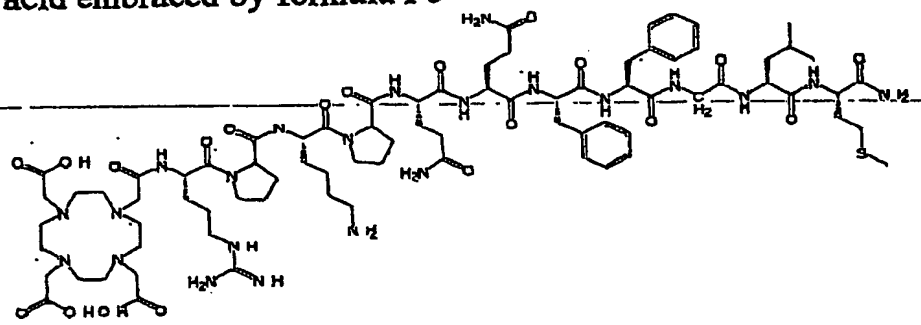


Ia

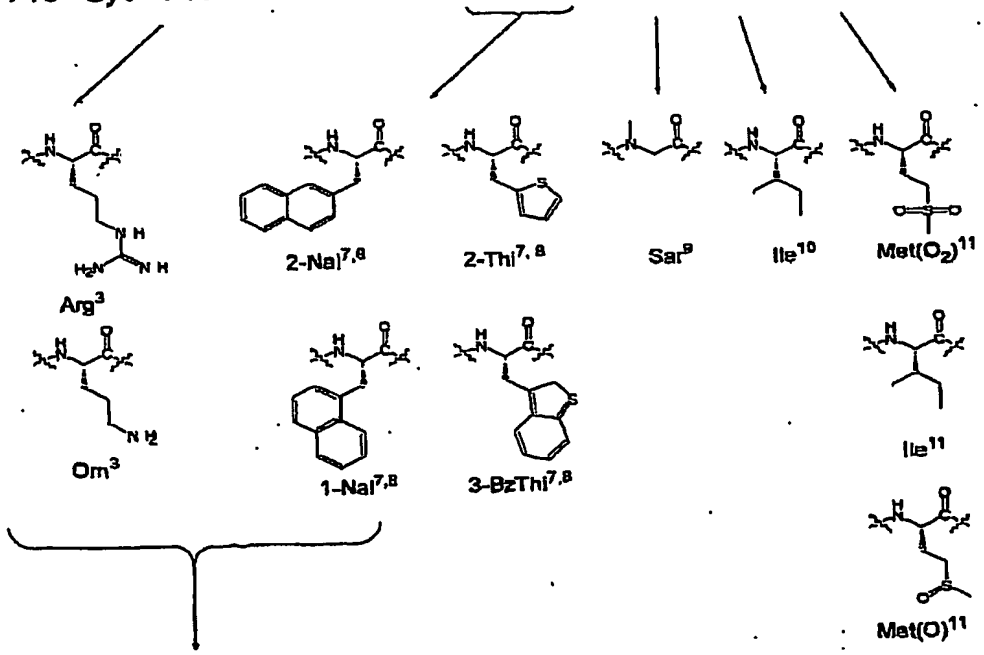
Legend: 2-Nal: 3-(2-naphthyl)-alanine; 1-Nal: 3-(1-naphthyl)-alanine; 2-Thi: 3-(2-thienyl)-alanine; Sar: sarcosine; Met(O): Methionine-sulfoxide; Met(O_2): Methionine-sulfone; 3-BzThi: 3-(3-benzothienyl)-alanine; Orn: ornithine; Ile: isoleucine.

wherein the substance P and analogue conjugates of formula Ia are labeled with metal isotopes selected from the group consisting of Actinium-225, Bismut-213, Cupper-64, Gallium-66, Gallium-68, Lutetium-177, Indium-111, Rhenium-186, Rhenium-188, Yttrium-86 and Yttrium-90.

Particularly the invention also relates to new radiolabeleed conjugates based on substance P and analogues thereof with the prochelator DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid embraced by formula I b



DOTA-Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH$_2$



I b

Legend: 2-Nal: 3-(2-naphthyl)-alanine; 1-Nal: 3-(1-naphthyl)-alanine; 2-Thi: 3-(2-thienyl)-alanine; Sar: sarcosine; Met(O): Methionine-sulfoxide; Met(O_2): Methionine-sulfone; 3-BzThi: 3-(3-benzothienyl)-alanine; Orn: ornithine; Ile: isoleucine.

wherein the substance P and analogue conjugates of formula I b are labeled with metal isotopes selected from the group consisting of Actinium-225, Bismut-213, Cupper-64, Gallium-66, Gallium-68, Lutetium-177, Indium-111, Rhenium-186, Rhenium-188, Yttrium-86 and Yttrium-90.

Especially important in this invention are radiolabeled conjugates of substance P and analogues selected from the group of undecapeptides:

a) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

b) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met(O_2)-NH_2,

c) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met-NH_2,

d) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Gly-Leu-Met-NH_2,

e) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met-NH_2

f) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met(O_2)-NH_2,

g) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Gly-Leu-Met(O_2)-NH_2,

h) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met(O_2)-NH_2,

i) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Sar-Leu-Met(O_2)-NH_2,

j) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met-NH_2,

k) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met(O_2)-NH_2

l) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met-NH_2,

m) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met(O_2)-NH_2,

n) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met-NH_2

o) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met(O_2)-NH_2

with the prochelator DOTAGA(tBu)4 (1-(1-carboxy-3-carboxytertbutoxypropyl)-4,7,10 (carboterbutoxymethyl)-1,4,7,10-tetraazacyclododecane; wherein the substance P and analogue conjugates mentioned under a) –o) are labeled with metal isotopes selected from the group consisting of Actinium-225, Bismut-213, Cupper-64, Gallium-66, Gallium-68, Lutetium-177, Indium-111, Rhenium-186, Rhenium-188, Yttrium-86 and Yttrium-90.

Especially important in this invention are radiolabeled conjugates of substance P and analogues selected from the group of undecapeptides:

a) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

b) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met(O_2)-NH_2,

c) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met-NH_2,

d) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Gly-Leu-Met-NH_2,

e) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met-NH_2

f) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met(O_2)-NH_2,

g) Arg-Pro-Lys-Pro-Gln-Gin-Phe-Thi-Gly-Leu-Met(O_2)-NH_2,

h) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met(O_2)-NH_2,

i) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Sar-Leu-Met(O_2)-NH_2,

j) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met,-NH_2,

k) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met(O_2)-NH_2

l) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met-NH_2,

m)) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met(O_2)-NH_2,

n) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met-NH_2,

o) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met(O_2)-NH_2,

with the prochelator DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid wherein the substance P and analogue conjugates mentioned under a) –o) are labeled with metal isotopes selected from the group consisting of Actinium-225, Bismut-213, Cupper-64, Gallium-66, Gallium-68, Lutetium-177, Indium-111, Rhenium-186, Rhenium-188, Yttrium-86 and Yttrium-90.

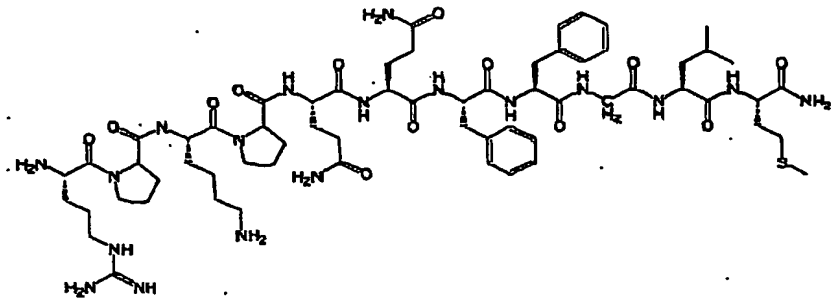
The radioconjugates of formula I, Ia, and Ib and the radioconjugates mentioned twice under a) – o) with different prochelators DOTAGA or DOTA are useful for diagnostic investigations using conventional scintigraphy with Indium-111 labeled substance P or analogues or using positron emission tomography with the tracers Gallium-68, Gallium-66, Yttrium-86 or Cupper-64. The diagnostic peptidic vector can either be applied locoregionally or systemically.

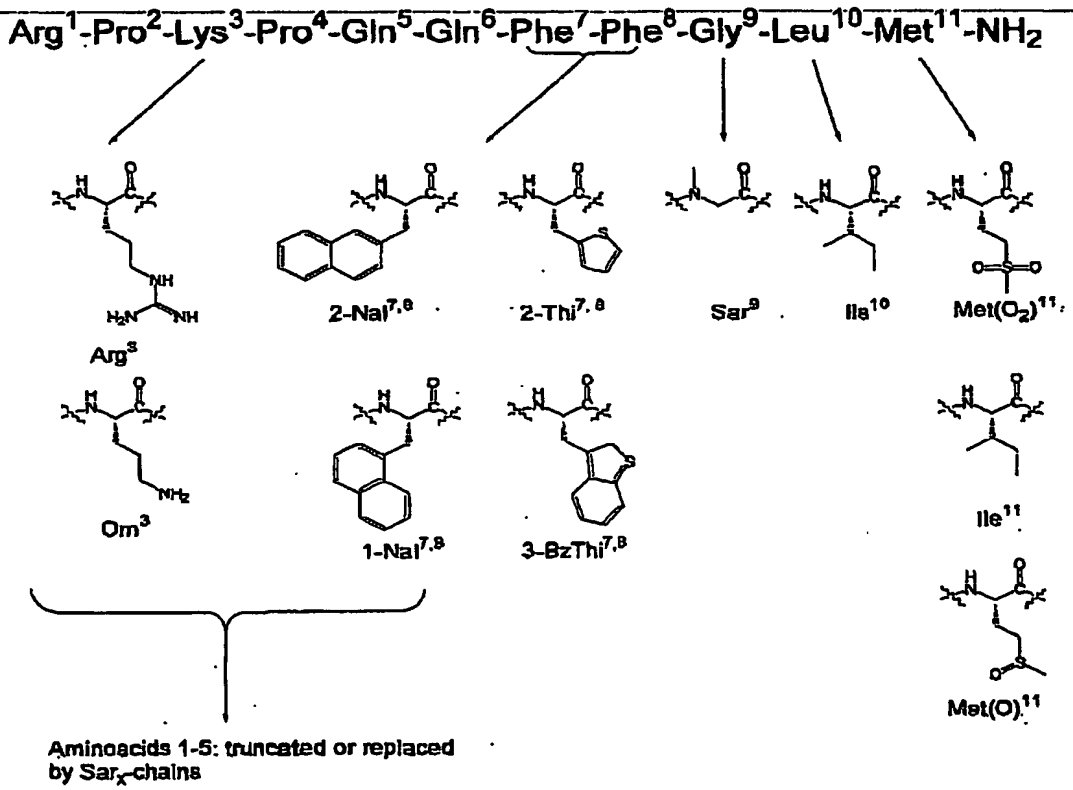
For therapeutic treatment the radioconjugates of formula I, Ia, Ib and the radioconjugates mentioned twice under a) –o) with different chelators DOTAGA or DOTA can be labeled with alpha-emitting isotopes, e.g. Bismuth-213, Actinium-225, and the beta-emitters Yttrium-90, Lutetium-177, Rhenium-188 and Rhenium-186.

Another aspect of the invention provides a method of targeting brain tumors and treating brain tumors in a host afflicted with brain tumors, e.g. gliomas. The method is especially useful in the detection and therapeutic treatment of brain tumors and satellite lesions thereof.

Therefore the invention is also relating to a method for the detection (targeting) and treatment of brain tumors, e.g. gliomas and the satellite lesions thereof, characterised in that the brain tumors,e.g. gliomas and the satellite lesions thereof to be targeted, locallized or therapeutically treated are brain tumors, e.g. gliomas.

A further embodiment of the invention are new substance P analogues , e.g. undecapeptides embraced by following formula II





II

Legend: 2-Nal: 3-(2-naphthyl)-alanine; 1-Nal: 3-(1-naphthyl)-alanine; 2-Thi: 3-(2-thienyl)-alanine; Sar: sarcosine; Met(O): Methione-sulfoxide; Met(O_2): Methionine-sulfone; 3-BzThi: 3-(3-benzothienyl)-alanine; Orn: ornithine; Ile: isoleucine.

with the exception of the undecapeptide compound of following formula Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (substance P) and Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met-NH_2 ([Sar^9]- substance P) or their methionine-sulfoxide (Met(O_2)) derivatives (substance P), being known compounds and as a further embodiment of this invention the process of manufacturing compounds of formula II.

Particularly important are substance P analogues as mentioned earlier as peptide part of the conjugate and are listed as follows:

a) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met-NH_2,

b) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Gly-Leu-Met(O_2)-NH_2,

c) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met(O_2)-NH_2,

d) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Sar-Leu-Met(O_2)-NH_2,

e) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met,-NH_2,

f) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met(O_2)-NH_2,

g) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met-NH_2,

h)) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met(O_2)-NH_2,

i) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met-NH_2,

j) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met(O_2)-NH_2,

The substance P analogues incorporated in the radiolabeled conjugates of formula I, Ia, Ib and the substance P anlogues per se of formula II are prepared following standard solid phase synthesis (SPPS) in a common side chain protected form as usually being the preferred version of synthesis.

The solid phase peptide synthesis (SPPS) is carried out for example on an Applied Biosystems Model using 431 A or 432 A Peptide synthesizer using

Fmoc (9-fluorenemethoxycarbonyl) strategy. The general principles and methods followed are well known in the art. For a more precise description it is referred to " Fluorenemethoxycarbonyl-polyamide solid phase synthesis-General Synthesis and Development" – Chapter 3 in "Solid peptide Synthesis – A practical approach" by E. Atherton and R.C. Sheppard, Information Press Ltd. Oxford , U.K. (1989)
For example , 9-fluorenemethoxycarbonyl (Fmoc) amino terminus protected amino acids are used. All the standard Fmoc-protected amino acids are purchased commercially. Coupling with dicyclohexylcarbodiimide/hydroxy-benzotriazol using for example either p-hydroxymethylphenoxy-methylpolystyrene for carboxyl terminus acids or Rink amide resin is used for carboxyl-terminus amides. When the synthesis is probably completed, the products are routinely cleaved using for example a solution comprised of trifluoracetic acid:water:anisole:triisopropylsilane or trifluoracetic acid: ethanedithiol: thioanisole:water for a few hours at room temperature. The obtained products are precipitaed by ether and purified by C-18 reverse phase chromatography.
The synthesis may be varied if necessary.

The obtained undecapeptide of formula II are coupled to the pochelators DOTAGA(^{t}Bu)$_4$ or DOTA. After cleavage and following deprotection DOTAGA-Substance P or DOTA-substance P and analogues were purified by preparative HPLC to a purity higher than 99,5%. The obtained substance P and analogue conjugates were labeled with a metal isotope.
The compounds of formula II , being substance P analogues are also useful in a method for targeting and treatment of brain tumors, e.g. gliomas as mentioned above for radiolabeled conjugates of formula I, Ia and Ib.

The invention includes the pharmaceutically acceptable salts and complexes of all the compounds of all the radiolabeled conjugates of formula I and Ia and the substance P analogues of formula II described herein.

The salts include, but are not limited to the following acids and bases. Examples of suitable inorganic acids include but are not limited to hydrochloric acid, hydrofluoric acid, hydrobromic acid, hydroiodic acid, sulfuric acid and boric acid. Examples of suitable organic acids include but are not limited to acetic acid, trifluoroacetic acid, formic acid, oxalic acid, malonic acid, succinic acid,, tartaric acid, maleic acid, fumaric acid, methanesulfonic acid, trifluoromethanesulfonic acid, benzoic acid, glycolic acid, lactic acid, citric acid and mandelic acid.

Examples of suitable inorganic bases include, but are not limited to ammonia, hydroxyethylamine and hydrazine. Examples of suitable organic bases include, but are not limited to methylamine, ethylamine, trimethylamine, triethylamine, ethylenediamine, hydroxyethylamine, morpholine, piperazine and guanidine.

The radiolabeled conjugates of formula I, formula Ia and the undecapeptides of formula II for targeting and treatment of brain tumors may be administered by every known route and may be selected from the group consisting of the intravenous route, the intraarterial route, the intracutaneus route, the subcutaneous route, the oral route, the buccal route, the intramuscular route, the anal route, the transdermal route, the intradermal route, the intrathecal route and the like.

In one preferred embodiment, the pharmaceutical carrier may be a liquid and the pharmaceutical composition would be in the form of a solution. In another equally preferred embodiment, the pharmaceutically acceptable composition is a solid and the pharmaceutical composition is in form of a powder or tablet or any other known solid formulation. In a further embodiment, the pharmaceutical carrier is a gel and the pharmaceutical composition is in the form of a suppository or cream. In a further embodiment, the compound may be formulated as part of a pharmaceutically acceptable transdermal patch.

Liquid carriers are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active ingredient can be dissolved or suspended in a pharmaceutically acceptable liquid carrier such as water, an organic solvent, a mixture of both, or pharmaceutically acceptable oils or fats. The liquid carrier can contain other suitable pharmaceutical additives such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agent, thickening agent, colors, viscosity regulators, stabilizer or osmo-regulators. Suitable examples of liquid carriers for oral and parenteral administration include water and other common additives.

Liquid pharmaceutical compositions, which are sterile solutions or suspensions can be utilized for intramuscuslar, intrathecal, intratracheal,, epidural, intraperitoneal or subcutaneaus injections. Sterile solutions can also be administered intravenously. The compounds may be prepared as a

sterile solid composition which may be dissolved or suspended (diffused) at the time of administration using sterile water, saline, or other appropriate sterile injectable medium.

Very much preferred is the local injection of diffusible peptidic vectors according to the invention for locoregional application.
The radiopharmaceutical is injected either into a stereotactically implanted ventricular catheter or into a capsule from which a catheter leads to the tumor center or into theresection cavity (also called port-a-cath-device).
A number of different,commercially available capsules and catheters can be used for this purpose.
In non-resected main tumor masses, the tip of the catheter has to be centered into the putative midportion of the tumor which can normally be achieved using a 3 dimensional stereotactic planning system. It is imporant to wait at least one day, preferably a few days, between catheter insertion and the first application in order to control the problem of back flow along the outside of the catheter away from the target site into the subarachnoid, subdural, epidural or subcutaneous space. This phenomenon can further be reduced by lowering the elevated intracranial pressure using osmodiuretics and high dose dexamethasone prior to application of the radiopharmakon. In solid non-resected cases, a slow infusion technique is used taking advantage of an infusion pump that allows continuous infusion of a volume of 5-10ml over a time period of 1-2 hours. This technique is distinct from the so called "convection enhanced delivery" of macromolecules into brain parenchyma that uses even slower infusion velocities to allow some penetration of large molecules that do not diffuse due to their size. Our radiopeptidic vector are virtually small drugs (1-2 kDaltons) and display highly diffusive properties. Diffusion across large areas of a tumor, even across the corpus callosum to the other hemisphere, has been repeatedly observed within 30 minutes following a bolus injection of 2-3 ml of the radiopharmaceutical.

The compounds of formulae I, Ia, Ib and II of the invention can be used alone or in combination with other pharmaceutically active substances. In the case of combinations with other pharmaceutically active compounds, a fixed combination of two or more components (e.g. kit of parts) are prepared as already known to a person of skill in the art, and the compound of the present invention and any other active compound are administered at an interval that allows common, additional or preferably synergistic effect for brain tumor and/or treatment, e.g.targeting and treatment of gliomas.

In any treatment regimen, the compositions of formula I, Ia, Ib or II may be administered, as mentioned above to a patient either singly or in a cocktail containing two or more targeted toxins, other therpeutic agents compositions, or the like, including, but not limited to, immunosuppressive agents, tolerance-inducing agents, potentiators and side-effect relieving agents- Particularly preferred agents useful in suppressing allergic reactions of a host. Preferred immunosuppressive agents include prednisone, DECADRON (Merck, Sharp and Dohme, West Point, Pa.), cyclophosphamide, cyclosporine, 6-mercaptopurine, methotrexate, azathioprine and i.v. gamma globulin or their combination. Preferred potentiators include monensin, ammonium chloride, perhexiline, verapamil amantadine and chloroquine. All of these agents are administered in generally-accepted efficacious dose ranges.

Optimal dosages to be administered may be determined by those skilled in the art, and will vary with the particular compound in use, the strength of the preparation, the mode of administration, and the advancement of the disease condition. Additional factors depending on the subject being treated, including subject age, weight, gender, diet and time of administration, will result in a need to adjust dosages for this special purpose. The administration of the compound may be effected continously or intermittently.

In the treatment, an appropriate dosage level will generally be about 0.001 to 50 mg/kg patient body weight per day which can be administered in single or multiple doses.
Preferably, the dosage level will be about 0.005 to about 25 mg/kg per day, more preferably about 0.01 to about 10 mg/kg per day, and even more preferably about 0.05 to about 1 mg/kg per day.

This invention is further illustrated in the Experimental Details Section which includes the Examples incorporated.

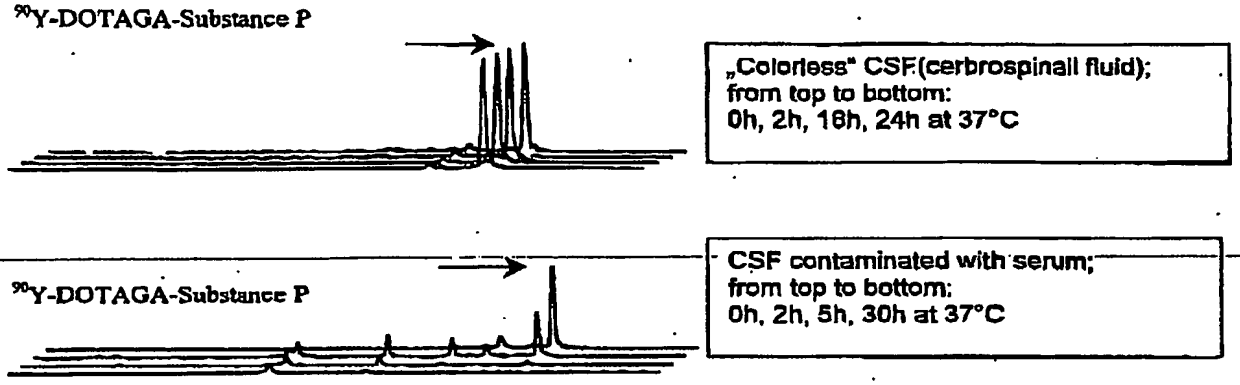
The following non limiting examples illustrate the inventor's preferred method for preparing and using the claimed compounds of the invention, which should not be construed as limiting the scope of this invention.

Experimental Details Section:

The radioconjugated substance P and analogues thereof being undeca-peptides are prepared following standard solid phase synthesis (SPPS) in a common side-chain protected form and the obtained peptide coupled to the prochelator DOTAGA(^{t}Bu)$_4$. After cleavage and following deprotection DOTAGA-Substance P and analogues were purified by preparative HPLC to a purity higher than 95%. The obtained substance P and analogue conjugates were sterilized and thereafter labeled with a metal isotope as mentioned above, e.g. $^{111}InCl_3$ (18mλ MBq per application) for imaging and $^{90}YCl_3$(37 MBq/µg DOTAGA-Substance P) using for example 0.4M sodium acetate buffer adjusted to pH 5.0 containing ascorbic acid (2g/10ml) and 2,5-dihydroxy benzoic acid (370 mg/19 ml).
The labeling could also be performed with ^{213}Bi, a very promising alpha emitter available from a Ac-225/Bi-213 generator. The labeling yields are in the same range as for Y-90 and In-111 (>95%), but Bi-213 has a short half-life time of only 47 min. This means the labeling process takes more than one half-life time and thus faster chelating agents would be more favourable. Further experiments on labeling shall be described in the experimental part (examples) thereafter.
During the labeling process a second peak has been identified on the HPLC chromatogram which was identified as $^{90}Y/^{111}In$-DOTAGA-[Met(O)^{11}]-Substance P as a product of radiolysis during the labeling process. Unfortunately this derivative showed a binding affinity to NK1 receptors by a factor 11 lower than the non-oxidized substance. Even by an addition of a large amount of an reducing agent, e.g. ascorbic acid to the buffer, the unwanted oxidation can not be surpressed completely. On the other side the synthesized derivative $^{90}Y/^{111}In$-DOTAGA-[Sar^9 Met(O_2)^{11}]-Substance P showed extremely higher affinity than the sulfoxide-derivatives (less than a factor of 2 lower than the native sequence). Thus it underlines that Met(O_2) at position 11 of the peptide sequence can be introduced without a significant loss of affinity and eliminates the eventually upcoming oxidation problem.

Further stability test by in vitro experiments incubating ^{90}Y-DOTAGA-Substance P in different CSF (cerbospinal fluid) aspirates from patients were executed.There a significant difference in degradation of the radiopeptide correlating with the "quality" of the CSF probe can be demonstrated. If the

aspirate is colorless (does not contain blood)of a clear aspect, the radioconjugate is stable for several hours, while in aspirates with a visible blood serum contamination, the substance was completely degraded within 5h.



This enzymatic degradation is also of high impact in patients, as seen in different urine samples from 0 to 24 h p.i. and CSF aspirates 1 week p.i.. All samples showed the same two radioactive metabolites as in the in vitro experiment with serum contaminated CSF probes. The quantification of the radioactivity in the urine also indicates a very fast wash-out of the applied ^{90}Y bound on metabolites of DOTAGA-Substance P.

Example 1

Synthesis of the Peptides

As already mentioned earlier peptides were synthesized on a solid phase using fmoc-strategy (fmoc = 9-Fluorenylmethoxycarbonyl-).
To achieve N-terminal amides, Rink amide MBHA resin was used. The synthesis were performed on a semiautomatic peptidesynthesizer from RINK Engineering, Winthertur, Switzerland. This synthesizer was equipped with one or up to 10 reaction vessels.
The aminoacids were purchased from Novabiochem AG, Läufelfingen, Switzerland. All other reagents were purchased from Fluka Chemie GmbH, Buchs, Switzerland.

In general, 1 equivalent of Rink-amide MBHA resin with a theoretical loading of 0.7 mmole/g resin was given to the reactor. Dimethylformamide (DMF) was added to the reactor and was shaked for 15 min to allow

swelling of the resin. After removing the solvent, again a portion of DMF was added for 2 min.
To the preswelled resin, a solution of 20% piperidine in DMF was added for 5 min to remove the fmoc-protection from the resin. This step was repeated once for 5 min and then again but for 12 min. After this, the solid phase was washed twice for 0.5 min with DMF and once for 1 min with DMF. The Piperidine-solutions and the DMF solutions of the last three washing were collected and filled with EtOH to 500 ml. From this solution a aliquot was taken to determine the amount of removed fmoc-protecting groups spectrophotometrically. Therefore, the absorbtion of the solution at 300 nm was determined and the original fmoc-amount was calculated.
Before coupling the fmoc-amino acid derivative to the solid phase, the resin was washed twice for 2 min with N-methyl-pyrolidinone (NMP).
Meanwhile, 3 equivalents of the fmoc-protected aminoacid derivative together with 3.3 equivalents of 1-Hydroxybenzotriazole and 3.3 equivalents of N,N'-Diisopropylcarbodiimide were dissolved in NMP and incubated for 45 min. Then, this coupling solution was added to the solid phase and the pH was adjusted to a value of 7-8 by adding about 5 equivalents of N,N-Diisopropylethylamine (DIPEA). The reaction was incubated for 90 min under gentle shaking.
After the reaction solution was removed, the solid phase was washed twice with DMF for 1 min.
The reaction was controlled performing a Kaiser-test: About 10 beads of the solid phase were washed 5 times with ethanol. 20 g phenol in 10 ml ethanol were mixed with 1ml of a solution of 1ml 0.01 M KCN in 49 ml pyridine. 50 µl of this mixture were added to the beads followed by 10 µl of a solution of 500 g ninhydrine in 10 ml ethanol. The beads were heated for 10 min to 95 °C. Blue beads indicate remaining free amino functions.
After attachment of the first amino acid, the following fmoc-amino acids were coupled in a similar manner but with a slightly different time program. The synthesizer was therefore programmed with following table:

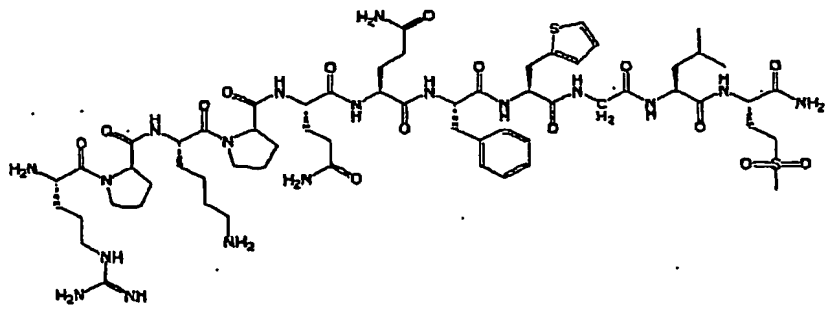
Step	Operation	Repetitions	Time	Collect waste
1	DMF	2	2	
2	PiperidinDMF	2	5	Yes
3	PiperidinDMF	1	9	Yes
4	DMF	2	0.5	Yes
5	DMF	1	1	Yes
6	NMP	2	2	
7	Reaction	1	90	
8	DMF	2	1	

All amino acids were used as N-terminal fmoc protected derivatives. Lysine was used with tert-butoxycarbonyl (Boc) protecting group on the side chain amino function. Arginine was used with 2,2,4,6,7-Pentamethyldihydro-benzofuran-5-sulfonyl (Pbf) protection of the side chain. If the peptide synthesis was performed in a single reactor, Kaiser test was performed after coupling of each amino acid. If Kaiser test indicated remaining free amino functions, the coupling of the amino acid was repeated.

After building of the whole peptide sequence, the solid phase was washed 5 times with isopropanol followed by 5 times washing with ether, each for 1 min. For 5 min, a constant air stream dried the solid phase.

Following peptides were synthesized:

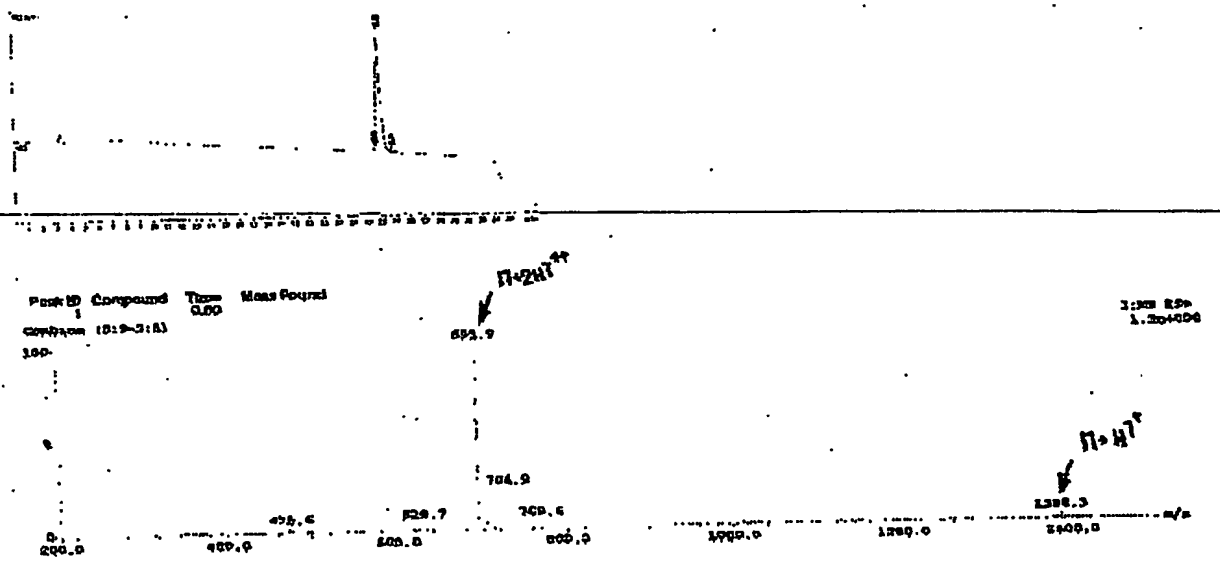
a)



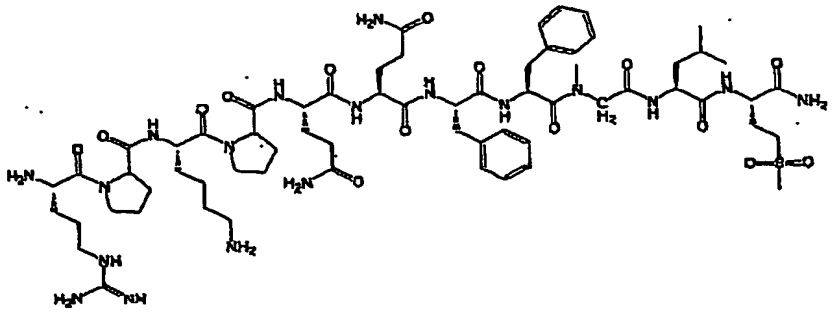
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Phe^{7}-Thi^{8}-Gly^{9}-Leu^{10}-$Met[O_2]^{11}$-NH_2

[Thi^8, Met(O$_2$)^{11}]-Substance P:

$C_{61}H_{96}N_{18}O_{15}S$
calculated (m/z): 1384.7
found: 1386.3 $(M+H)^+$, 693.9 $(M+2H)^{2+}$.



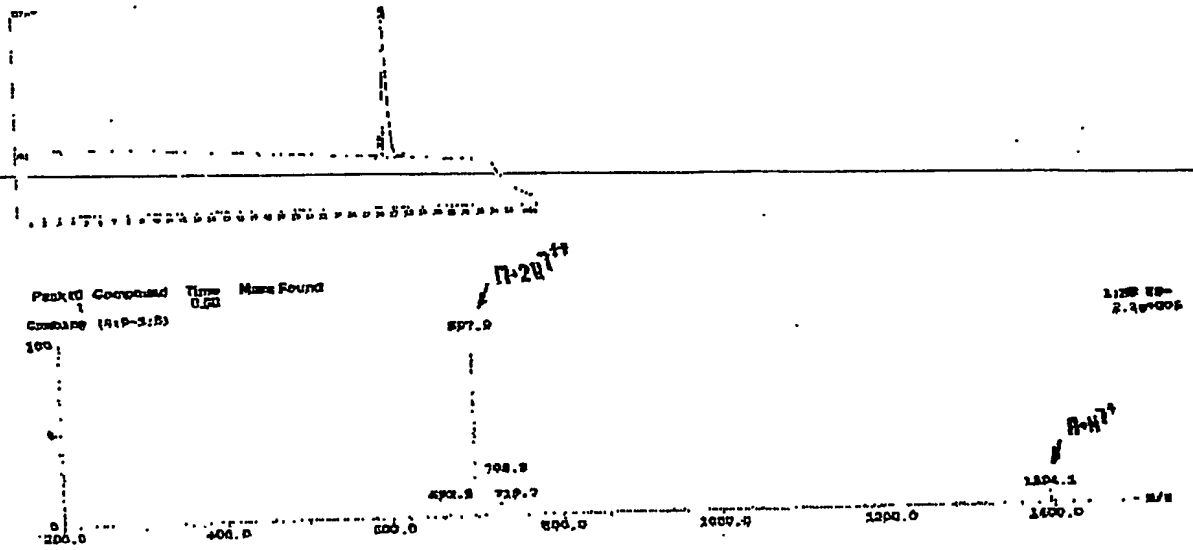
b)



Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Sar^9-Leu^{10}-Met[O$_2$]^{11}-NH$_2$

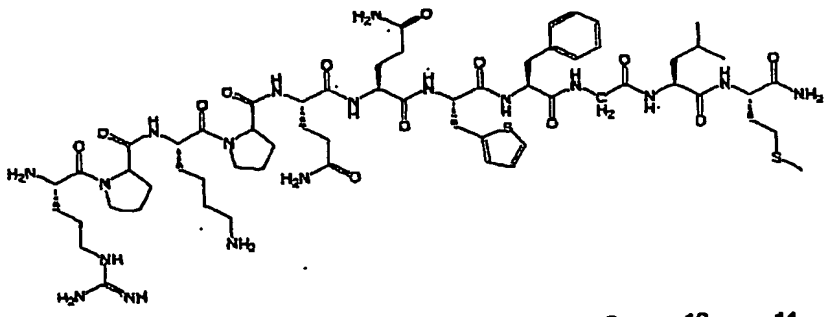
Sar^9, Met(O$_2$)^{11}]-Substance P:

$C_{64}H_{100}N_{18}O_{15}S$
calculated (m/z): 1392.7
found: 1394.1 $(M+H)^+$, 697.9 $(M+2H)^{2+}$.



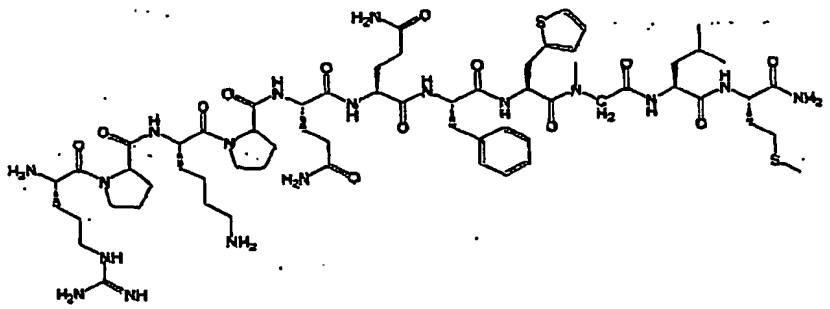
The following peptides synthesized were used as intermediates for preparing the conjugates directly:

c)



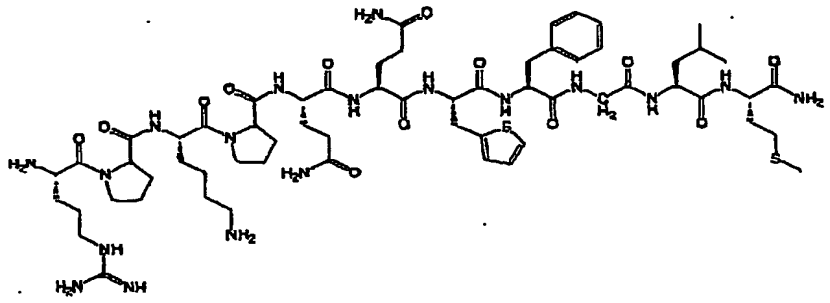
Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Thi^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH_2

d)



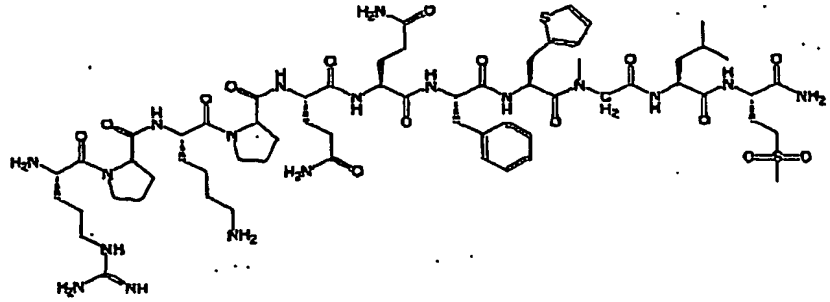
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Phe^{7}-Thi^{8}-Sar^{9}-Leu^{10}-Met^{11}-NH_2

e)



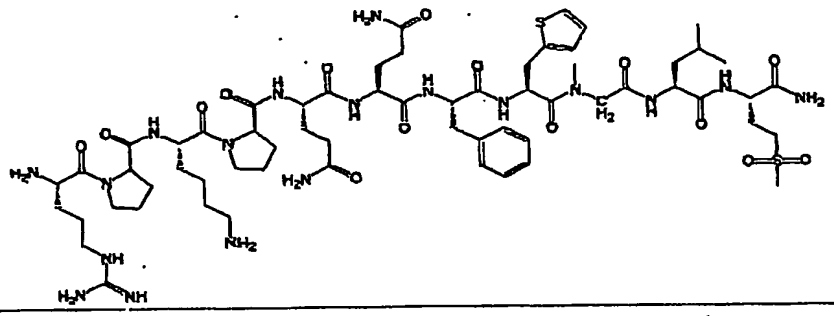
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Thi^{7}-Phe^{8}-Gly^{9}-Leu^{10}-Met^{11}-NH_2

f)



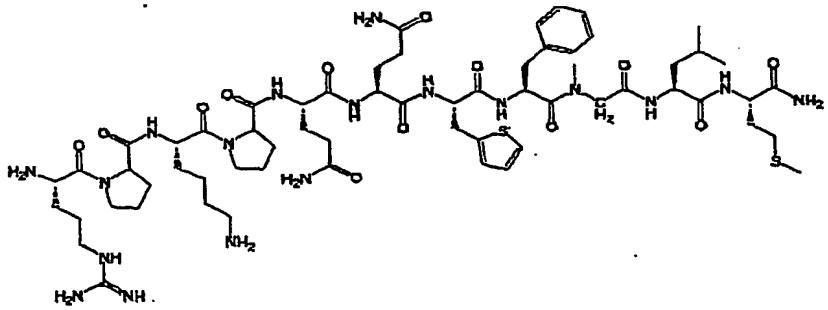
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Phe^{7}-Thi^{8}-Sar^{9}-Leu^{10}-$Met[O_2]^{11}$-NH_2

g)



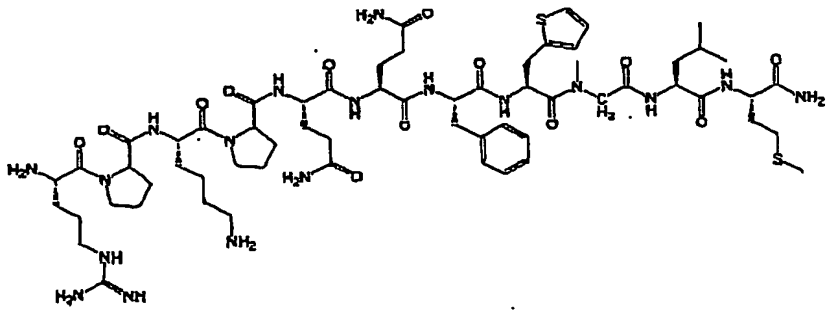
Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Thi^8-Sar^9-Leu^{10}-$Met[O_2]^{11}$-NH_2

h)



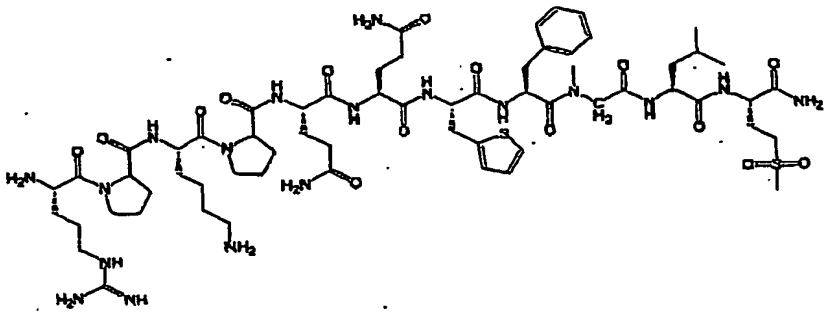
Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Thi^7-Phe^8-Sar^9-Leu^{10}-Met^{11}-NH_2

i)



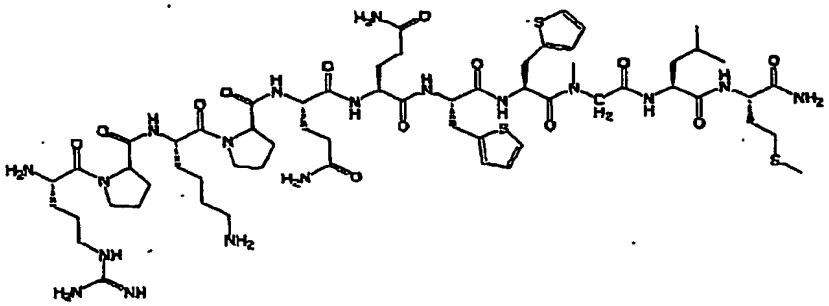
Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Thi^8-Sar^9-Leu^{10}-Met^{11}-NH_2

j)



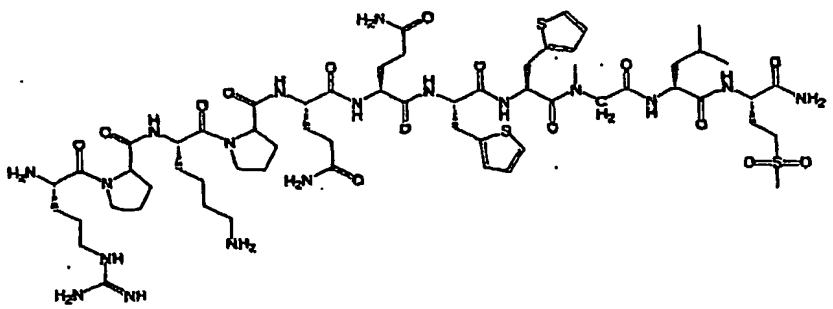
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Thi^{7}-Phe^{8}-Sar^{9}-Leu^{10}-Met[O$_2$]^{11}-NH$_2$

k)



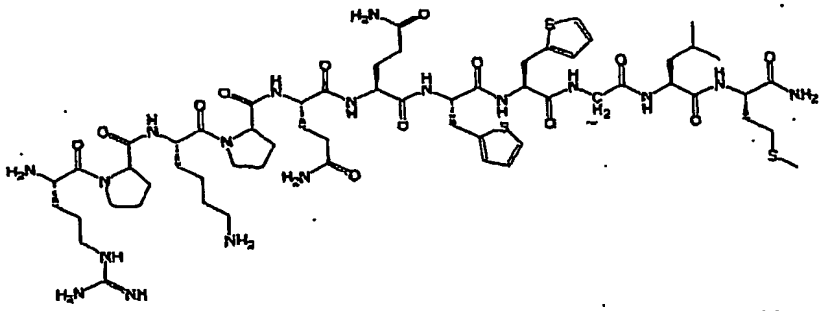
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Thi^{7}-Thi^{8}-Sar^{9}-Leu^{10}-Met^{11}-NH$_2$

l)



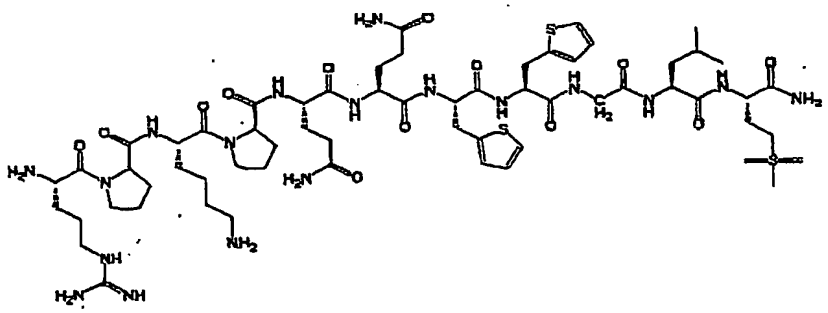
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Thi^{7}-Thi^{8}-Sar^{9}-Leu^{10}-Met[O$_2$]^{11}-NH$_2$

m),



Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Thi^7-Thi^8-Gly^9-Leu^{10}-Met^{11}-NH_2

n)



Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Thi^7-Thi^8-Gly^9-Leu^{10}-$Met[O_2]^{11}$-NH_2

a) Coupling of Prochelators

The prochelator DOTAGA(^{t}Bu)$_4$ was synthesized as described by K.-P. Eisenwiener et al, Bioorg med chem lett, **10** (2002) 2133 – 2135 or PCT/EPO1/05483 (H.R. Mäcke et al). DOTA(^{t}Bu)$_3$ was purchased at Macrocyclics Inc., Dallas, USA. Prior coupling the Prochelator, the N-terminal fmoc-protection was removed from the resin bound peptide. Therefore, it was swelled for 15 min in DMF, washed again for 2 min with DMF and treated then twice with a solution of 20% piperidin in DMF for 5

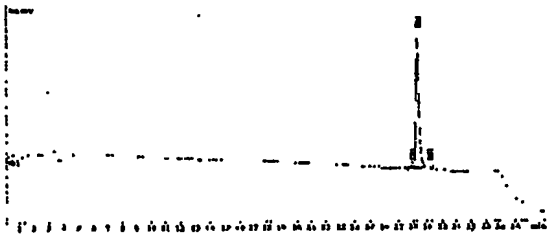
min. Then, the solid phase was treated another 12 min with this solution and washed twice 0.5 min with DMF and for another minute with DMF.
The solution from the piperidin treatments and the following DMF washings were collected to determine the content of cleaved fmoc groups.
2 equivalents of Prochelator and 1.2 equivalents of O-(7-Azabenzotriatol-1-yl)-N,N,N'N'-tetramethyluronium hexafluorophosphate (HATU) were dissolved in DMF and incubated for 45 min. After this, 2 equivalents of DIPEA were added to the solution. This reaction solution was added then to the solid phase and after 2 min the pH of the reaction was controlled and, if needed, adjusted to a value between 7 – 8 by adding some more DIPEA. The reaction was shaked over night.
After removing the solution, the solid phase was washed twice with DMF for 2 min, 5 times with isopropanol and 5 times with ether for 1 min. It was dried in a air stream for 10 min.

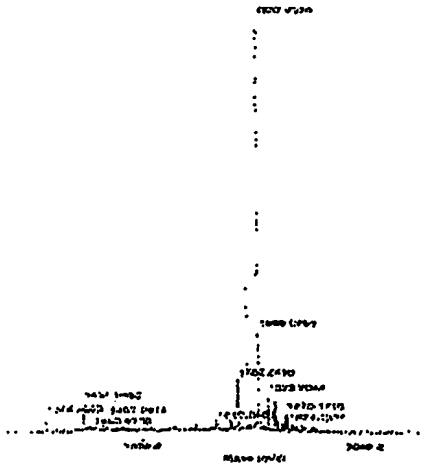
b) Deprotection, cleavage and purification

The solid phase was given to a syringe equipped with a frit. A solution of 91% trifluoroacetic acid (TFA), 5% thioanisole, 3% water and 1% triisoproylsilane was added and the syringe was agitated for 1 h. The solution was then poured to a polypropylene tube. To this solution was added a mixture of 50% diisopropylether and 50% petrolether. For 30 min the tube was cooled at –20°C. The precipitation was collected by centrifugation of the tube at 1006 g for 5 min and the solvent was decanted. Meanwhile, the solid phase in the syringe was treated again with the upper deprotection solution for 2 h. The deprotection solution was added to the already achieved precipitation and the solid was diluted with this. After another 3 h of deprotection, the crude product was precipitated again, cooled, centrifuged and separated from the solvent by decantation. The crude product was kept then at a vacuum (<100 mbar) over night to remove the remaining ethers.
The crude product was dissolved in a mixture of acetonitrile : water = 1 : 1 and purified by preparative HPLC on a Macherey-Nagel VP Nucleosil 100-5 C18, 21x250 mm. The preformed eluent gradient is following: A = acetonitrile, B = 0.1% TFA in water; flow = 15 ml/min; 0 min, 15% A; 25 min, 50% A; 26 min, 100% A; 28 min, 100% A; 30 min, 15% A. The main peak was collected and evaporated to dryness. It was analyzed by analytical HPLC and mass spectroscopy (MALDI-TOF-MS).

HPLC-chromatograms and MS-spectra:

1) DOTAGA-Substance P:
$C_{82}H_{128}N_{22}O_{22}S$, calculated (m/z): 1806.1, found1807.2

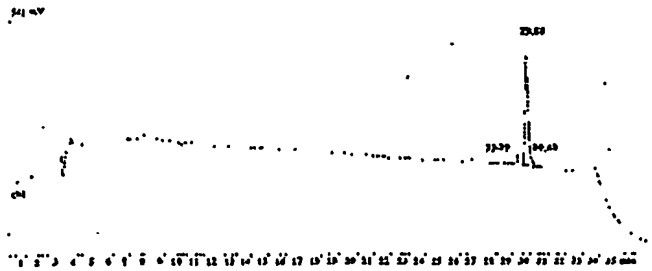


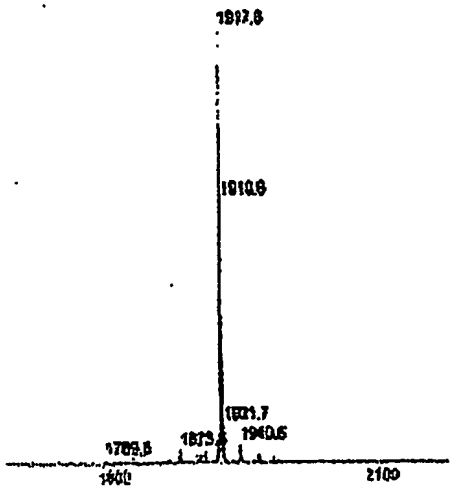


HPLC-chromatograms and MS-spectra:

2) In-DOTAGA-Substance P:

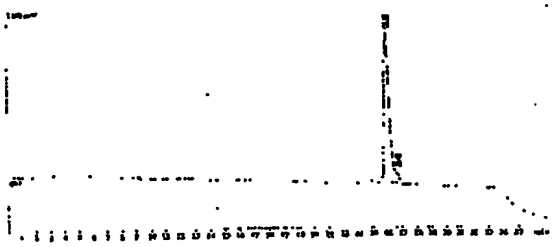
$C_{82}H_{125}InN_{22}O_{22}S$
calculated (m/z): 1916.81, found:1917.6 $(M+H)^+$.

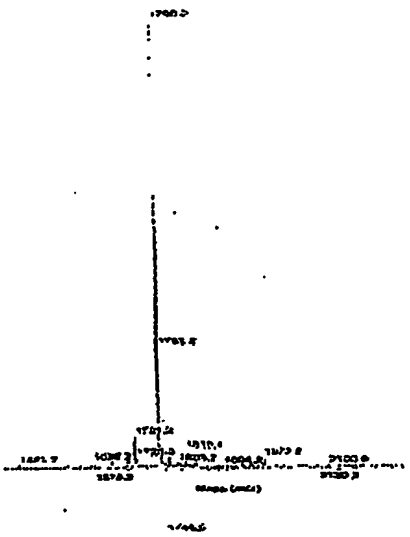




3) DOTA-[Met$(O_2)^{11}$]-Substance P:

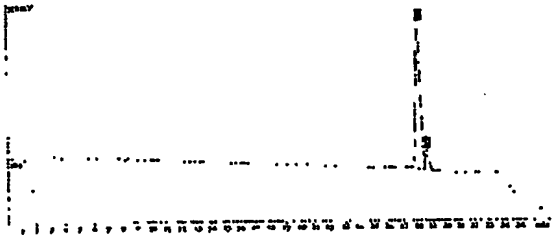
$C_{79}H_{126}N_{22}O_{22}S$, calculated (m/z): 1766.9, found 1766.

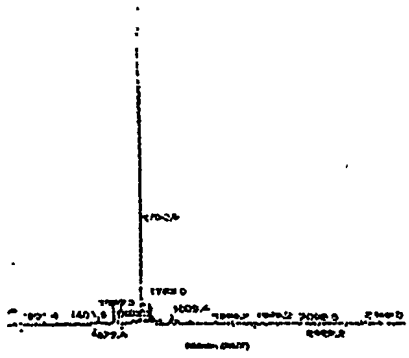




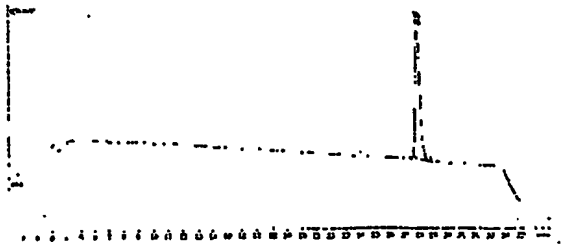
4) DOTA-[Sar^9]-Substance P:

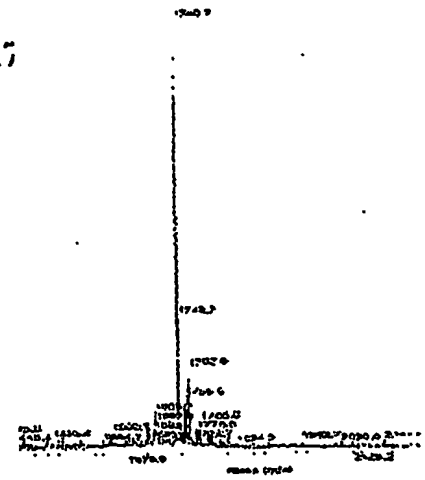
$C_{80}H_{128}N_{22}O_{22}S$, calculated (m/z): 1748.9, found 1748.5





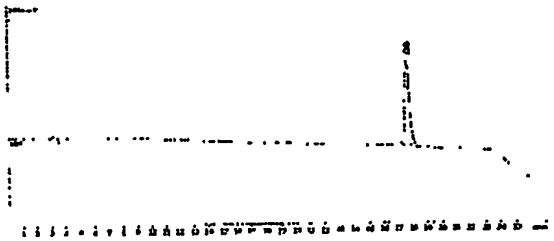
5) DOTA-[Thi^8]-Substance P:
$C_{77}H_{124}N_{22}O_{20}S_2$, calculated (m/z): 1740.9, found 1740.7

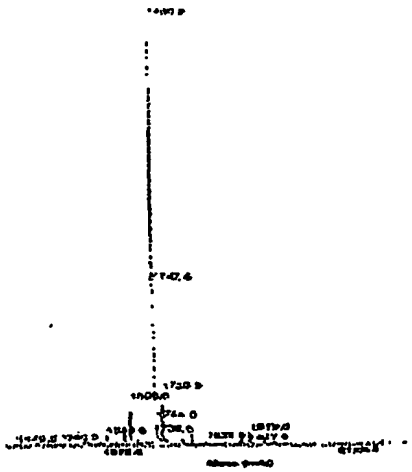




6) DOTA-[Thi^{7}]-Substance P:

$C_{77}H_{124}N_{22}O_{22}S_2$, calculated (m/z): 1740.9, found 1740.6



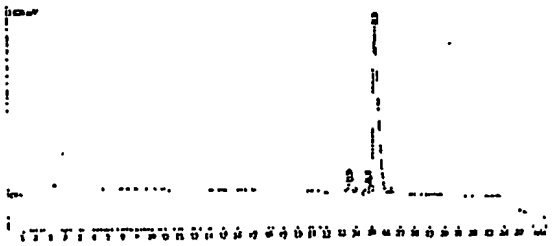


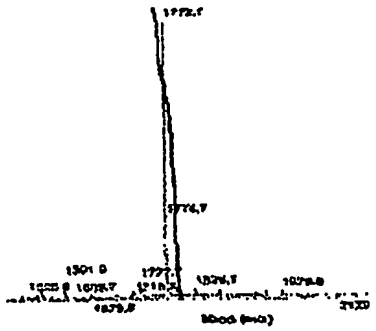
7) DOTA-[Sar^{9}, Met(O$_2$)^{11}]-Substance P:

$C_{80}H_{128}N_{22}O_{22}S$, calculated (m/z): 1780.9, found 1780.1

8) DOTA-[Thi^{8}, Met(O$_2$)^{11}]-Substance P:

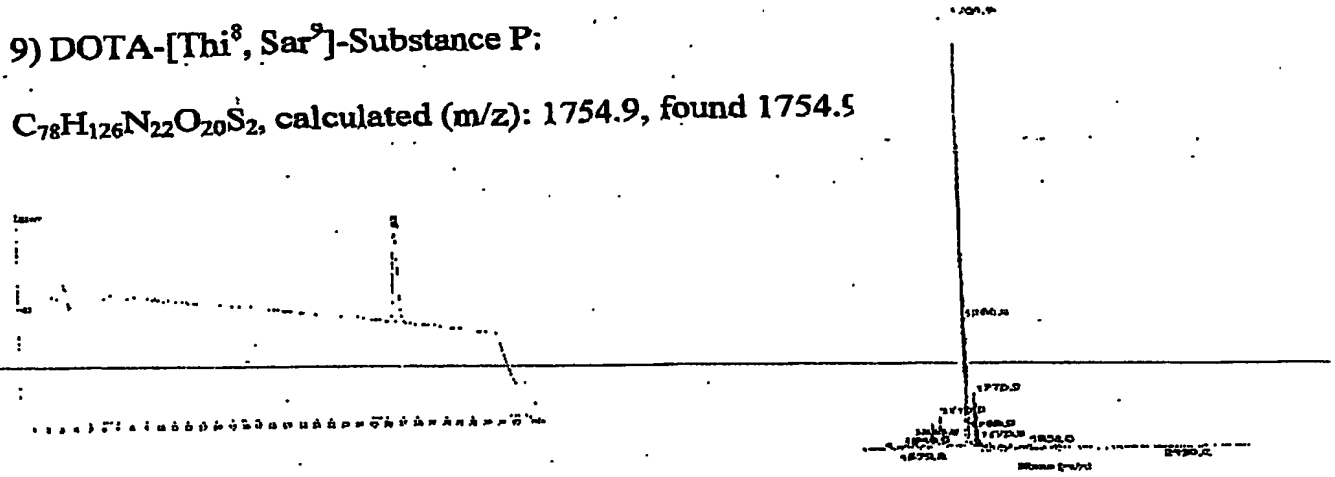
$C_{77}H_{124}N_{22}O_{22}S_2$, calculated (m/z): 1772.9, found 1772.7





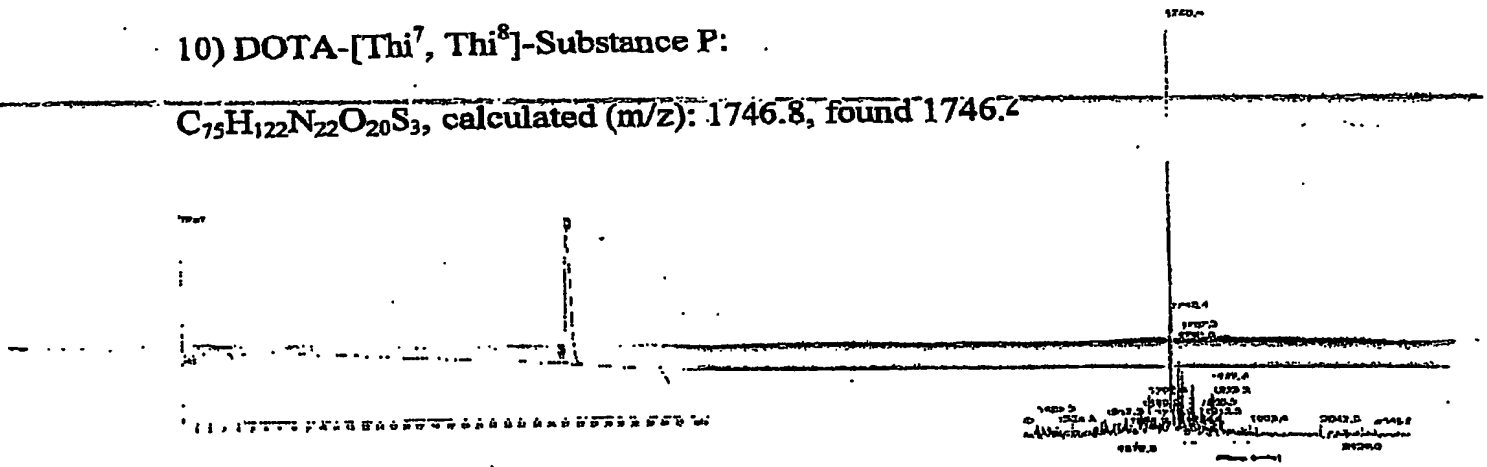
9) DOTA-[Thi^8, Sar^9]-Substance P:

$C_{78}H_{126}N_{22}O_{20}S_2$, calculated (m/z): 1754.9, found 1754.5



10) DOTA-[Thi^7, Thi^8]-Substance P:

$C_{75}H_{122}N_{22}O_{20}S_3$, calculated (m/z): 1746.8, found 1746.2



c) Labeling

An aliquot of the peptide-chelator conjugate was dissolved in water and a 0.4 M sodium acetate buffer (pH 5) was added. To this solution 10 equivalents of an aqueous solution of $InCl_3$*5 H_2O was added and incubated for 1 h at 95 °C. After cooling to room temperature, it was purified over a SepPak C_{18} cartridge preconditioned with 10 ml methanol and 20 ml water. The cartridge was eluted with 10 ml of 0.4 M sodium acetate (pH 5)

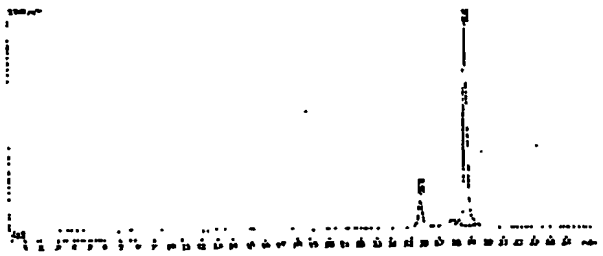
followed by 5 ml of methanol. The methanol phase was evaporated to dryness to afford the Indium-chelator-peptide complex.
The same procedure was also performed for other metals.

d) Radioactive labeling

This is the same procedure as for non-radioactive labeling, but with excess of Chelator-peptide to obtain an optimal labeling yield. Excess is dependent on the used metal.
For ^{90}Y-/^{111}In-DOTAGA-Substance P: To a sterile 30 µg Aliquot of the Chelator-peptide conjugate 30 mCi ^{90}Y-solution and 0.5 mCi ^{111}In-solution were added and filled up to 500 µl with sterile 0.4 M NaOAc-Buffer (pH 5). This solution was heated for 30 min to 95°C and cooled to room temperature for 15 min. The solution was diluted to 1 ml with physiological NaCl-solution. A sample of it is controlled on the radio-HPLC for determining the content of non-bound radiometal.

Typical Radio-chromatogram after labeling of 30 µg of DOTAGA-Substance P with 30 mCi of ^{90}Y and 0.5 mCi of ^{111}In:

(first peak correspond to ^{90}Y-DOTAGA-[Met(O)^{11}]-Substance P, second peak correspond to ^{90}Y-DOTAGA-Substance P)



Example 3

Receptor autoradiography in primary brain tumors comparing the somatostatin an the neurokinin-1 (NK-1) targeting system

An immunohistochemical study of representative cases of primary gliomas conducted, showed that the distinction between tumor infiltrated brain tissue and normal brain is, in many instances, virtually impossible because of the background expression of somatostatin receptors on normal brain tissue. Despite this observation, In-111 DOTATOC scintigrams show confinement of the injected radiopharmakon to the tumor compartment as visualized on MRI. This might best be explained by the chaotic structure of the extracellular tissue architecture within the tumor and the infiltrated brain as compared to non-infiltrated brain tissue. Bakay has shown in his publication (Brain 1970, 93, 693-698) that the extracellular space can make up 20-40% of a given volume of brain tumor tissue as compared to normal brain which contains about 5% of extracellular space. Physically speaking, a tumor utilizes the available energy to proliferate and infiltrate, and not to maximize the order of the molecular architecture as normal brain tissue does (law of entropia). It has been described the consistant and high expression of neurokinin-1 receptors in glioblastomas, but also in low- and intermediate grade gliomas. Accordingly a comparative autoradiographic study between the somatostatin/DOTATOC and the substance P/NK-1 system has been conducted in order to define the best targeting compounds for locoregional therapy in brain tumors. With one single exception of an ependymoma, the expression of NK-1 receptors was at least equivalent or higher in most gliomas of grades II-IV. The results teach that the substance P/NK-1 system can replace the somatostatin/DOTATOC system easily yielding superior responses due to a clearly more favorable tumor:brain background ratio.

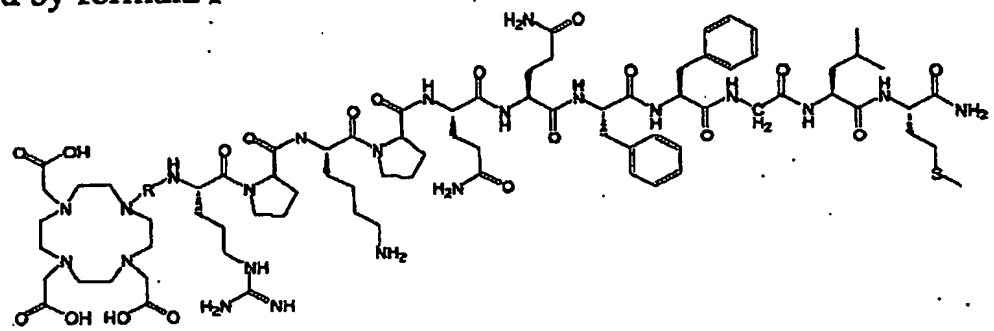
Example 4

In the following substance P pilot study, 4 cases with biopsy alone followed by Y-90-DOTAGA substance P (treatment) were included; all cases with deep or eloquently located tumors where open surgery did not seem advisable as a first treatment option and 5 cases with open gross total resection and subsequent radiopeptide brachytherapy. As a third group which can serve as a control, 5 advanced cases that had been treated by the

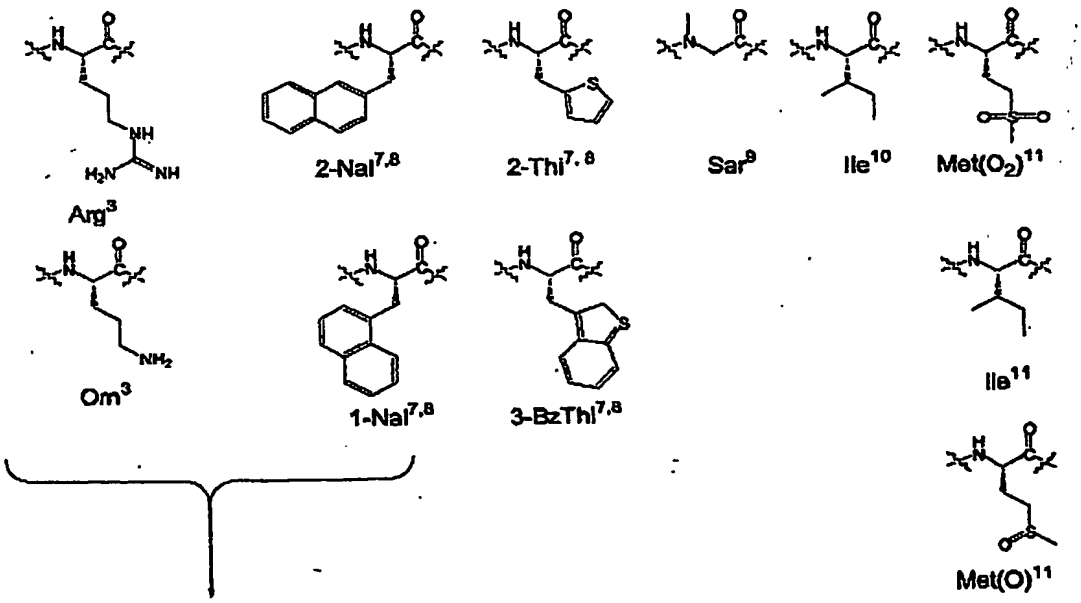
so called standard regimens with virtually no therapeutic option left anymore were included.
Since this is a non-controlled pilot study, it had to be relied on the results of the only randomized large glioma trial executed and published more than 20 years ago (Walker et al, 1980, N Engl J Med). In cases with biopsy alone, average survival is around 9 weeks without further treatment. In the 4 cases of this pilot study, all patients survived at least 2 months and up to 9 months, and in the special case W.M. even showed a remarkable partial response. In the resected 5 cases followed by internal radiopeptide brachytherapy, overall survival appeared to be longer than expected which was in the range between 14 and 26 months. According to the only large randomized trial of 1970s which serves as historical control, resection alone would have led to an expected survival of about 4 months, and resection plus an external beam radiotherapy to a mean survival of about 9.5 months. Quite remarkably, all survival numbers are located on the right of these expected values on the time axis which can be taken as strong indication that the conjugate Y-90DOTAGA substance P is certainly more efficient than a pure water solution.
This interpretation of the data is further supported by an impressive partial response (case W.M.) which was obtained in a non-resectable left-sided thalamic glioblastoma. A key question in local glioma therapy is whether one can achieve to reach infiltrating tumor cell nests or satellite lesion. In an illuminating case, diffusibility was shown in a 4 cm distant satellite lesion that becomes visible following intracavitary injection into there section cavity. Despite good labeling, the satellite lesion could not be controlled with Y-90 substance P. This case proves that even if the target can be labeled, a radionuclide with a narrower range would have been much more likely to eradicate this lesion.
In contrast the presently alpha-emitters (µm range) and narrow range beta-emitters (e.g. Lu-177, 1-2 mm) should prove superior in the treatment of nivasive tumor cell clusters to the presently used dissipative Y-90 (range 3-6 mm) is more suitable for bulkier lesions.

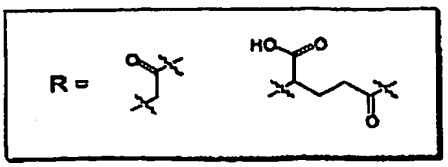
Sequence Listing / KBS 101

1) New radiolabeled conjugates based on substance P and analogues thereof with the prochelator and subsequent chelator DOTAGA(tBu)4 (1-(1-carboxy-3-carboxytertbutoxypropyl)-4,7,10 (carbotertbutoxymethyl)-1,4,7,10-tetraazacyclododecane or the chelator DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid embraced by formula I



Chelator-Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH$_2$



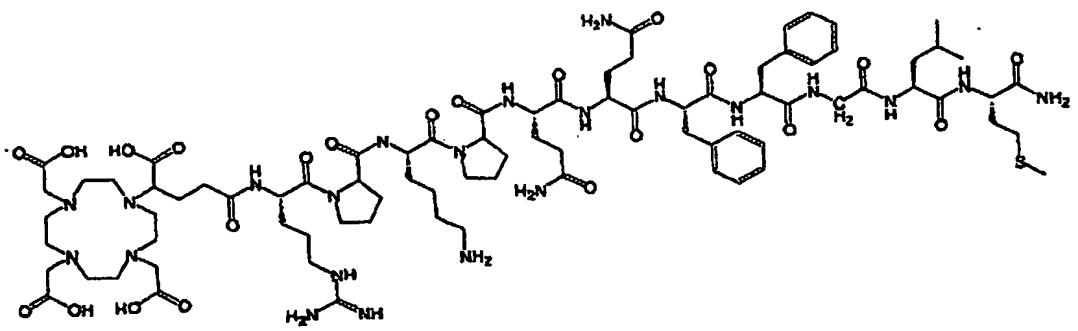


I

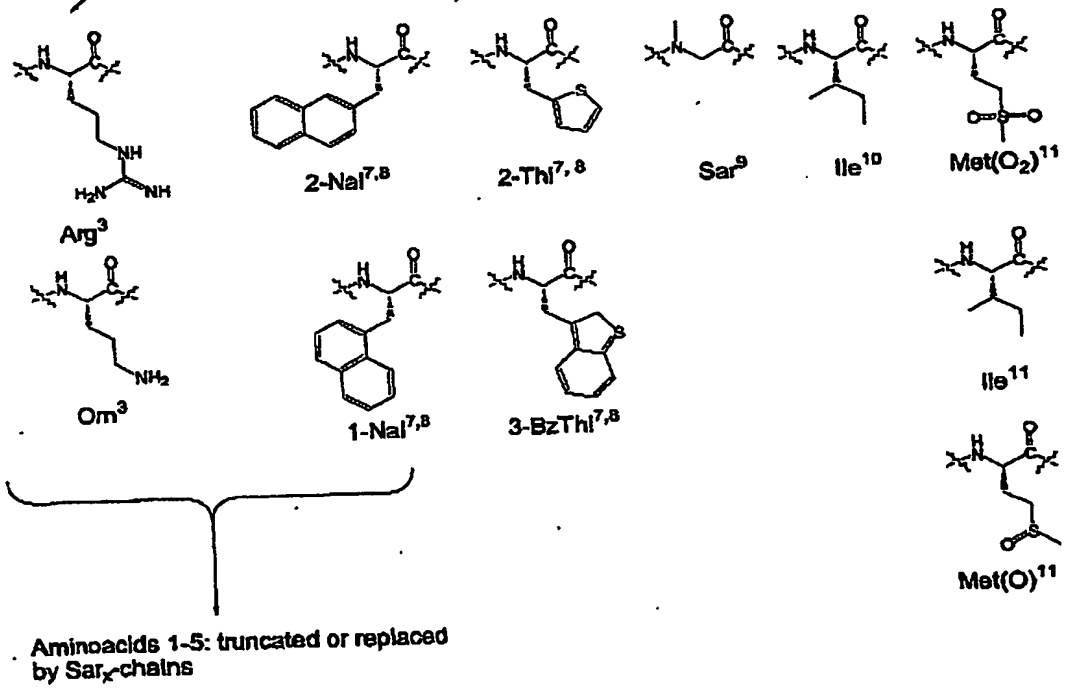
Legend: 2-Nal: 3-(2-naphthyl)-alanine; 1-Nal: 3-(1-naphthyl)-alanine; 2-Thi: 3-(2-thienyl)-alanine; Sar: sarcosine; Met(O): Methionine-sulfoxide; Met(O_2): Methionine-sulfone; 3-BzThi: 3-(3-benzothienyl)-alanine; Orn: ornithine; Ile: isoleucine.

wherein the substance P and anlogue conjugates of formula I are labeled with metal isotopes selected from the group consisting of Actinium-225, Bismut-213, Cupper-64, Gallium-66, Gallium-68, Lutetium-177, Indium-111, Rhenium-186, Rhenium-188, Yttrium-86 and Yttrium-90 or the like known for radiolabeling.

2) New radiolabeled conjugates based on substance P and analogues thereof with the prochelator and subsequent chelator DOTAGA(tBu)4 (1-(1-carboxy-3-carboxytertbutoxypropyl)-4,7,10 (carbotertbutoxymethyl)-1,4,7,10-tetraazacyclododecane of formula Ia



DOTAGA-Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Phe^{7}-Phe^{8}-Gly^{9}-Leu^{10}-Met^{11}-NH$_2$

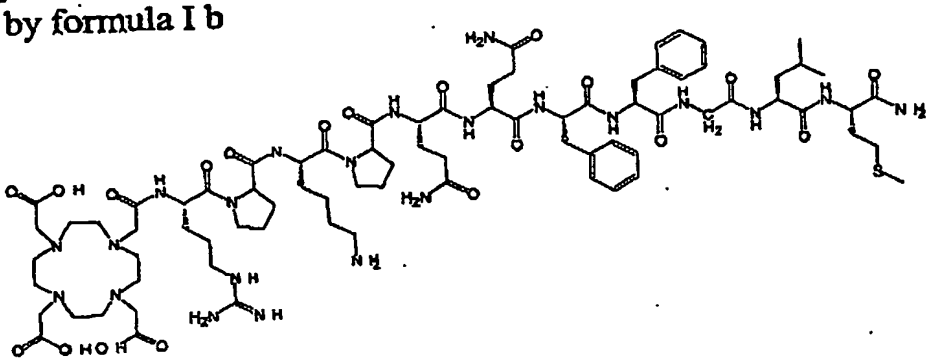


Ia

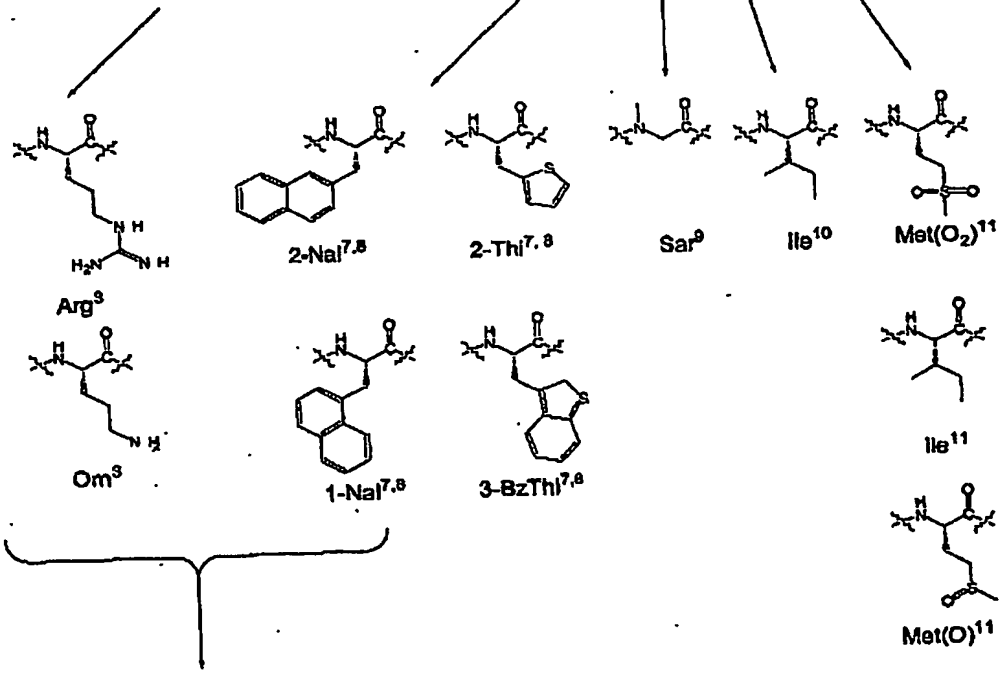
Legend: 2-Nal: 3-(2-naphthyl)-alanine; 1-Nal: 3-(1-naphthyl)-alanine; 2-Thi: 3-(2-thienyl)-alanine; Sar: sarcosine; Met(O): Methionine-sulfoxide; Met(O$_2$): Methionine-sulfone; 3-BzThi: 3-(3-benzothienyl)-alanine; Orn: ornithine; Ile: isoleucine.

wherein the substance P and analogue conjugates of formula Ia are labeled with metal isotopes selected from the group consisting of Actinium-225, Bismut-213, Cupper-64, Gallium-66, Gallium-68, Lutetium-177, Indium-111, Rhenium-186, Rhenium-188, Yttrium-86 and Yttrium-90.

3) new radiolabeleed conjugates based on substance P and analogues thereof with the prochelator and subsequent chelator DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid embraced by formula I b



DOTA-Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH$_2$



I b

Legend: 2-Nal: 3-(2-naphthyl)-alanine; 1-Nal: 3-(1-naphthyl)-alanine; 2-Thi: 3-(2-thienyl)-alanine; Sar: sarcosine; Met(O): Methionine-sulfoxide; Met(O_2): Methionine-sulfone; 3-BzThi: 3-(3-benzothienyl)-alanine; Orn: ornithine; Ile: isoleucine.

wherein the substance P and analogue conjugates of formula I b are labeled with metal isotopes selected from the group consisting of Actinium-225, Bismut-213, Cupper-64, Gallium-66, Gallium-68, Lutetium-177, Indium-111, Rhenium-186, Rhenium-188, Yttrium-86 and Yttrium-90.

4) New are radiolabeled conjugates of substance P and analogues selected from the group of undecapeptides:

a) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

b) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met(O_2)-NH_2,

c) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met-NH_2,

d) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Gly-Leu-Met-NH_2,

e) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met-NH_2

f) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met(O_2)-NH_2,

g) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Gly-Leu-Met(O_2)-NH_2,

h) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met(O_2)-NH_2,

i) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Sar-Leu-Met(O_2)-NH_2,

j) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met-NH_2,

k) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met(O_2)-NH_2

l) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met-NH_2,

m) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met(O_2)-NH_2,

n) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met-NH_2

o) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met(O_2)-NH_2

with the prochelator and subsequent chelator DOTAGA(tBu)4 (1-(1-carboxy-3-carboxytertbutoxypropyl)-4,7,10 (carboterbutoxymethyl)-1,4,7,10-tetraazacyclododecane, wherein the substance P and analogue conjugates mentioned under a) –o) are labeled with metal isotopes selected from the group consisting of Actinium-225, Bismut-213, Cupper-64, Gallium-66, Gallium-68, Lutetium-177, Indium-111, Rhenium-186, Rhenium-188, Yttrium-86 and Yttrium-90.

5) New radiolabeled conjugates of substance P and analogues selected from the group of undecapeptides:

a) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

b) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met(O_2)-NH_2,

c) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met-NH_2,

d) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Gly-Leu-Met-NH_2,

e) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met-NH_2

f) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met(O_2)-NH_2,

g) Arg-Pro-Lys-Pro-Gln-Gin-Phe-Thi-Gly-Leu-Met(O_2)-NH_2,

h) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met(O_2)-NH_2,

i) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Sar-Leu-Met(O_2)-NH_2,

j) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met,-NH_2,

k) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met(O_2)-NH_2

l) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met-NH_2,

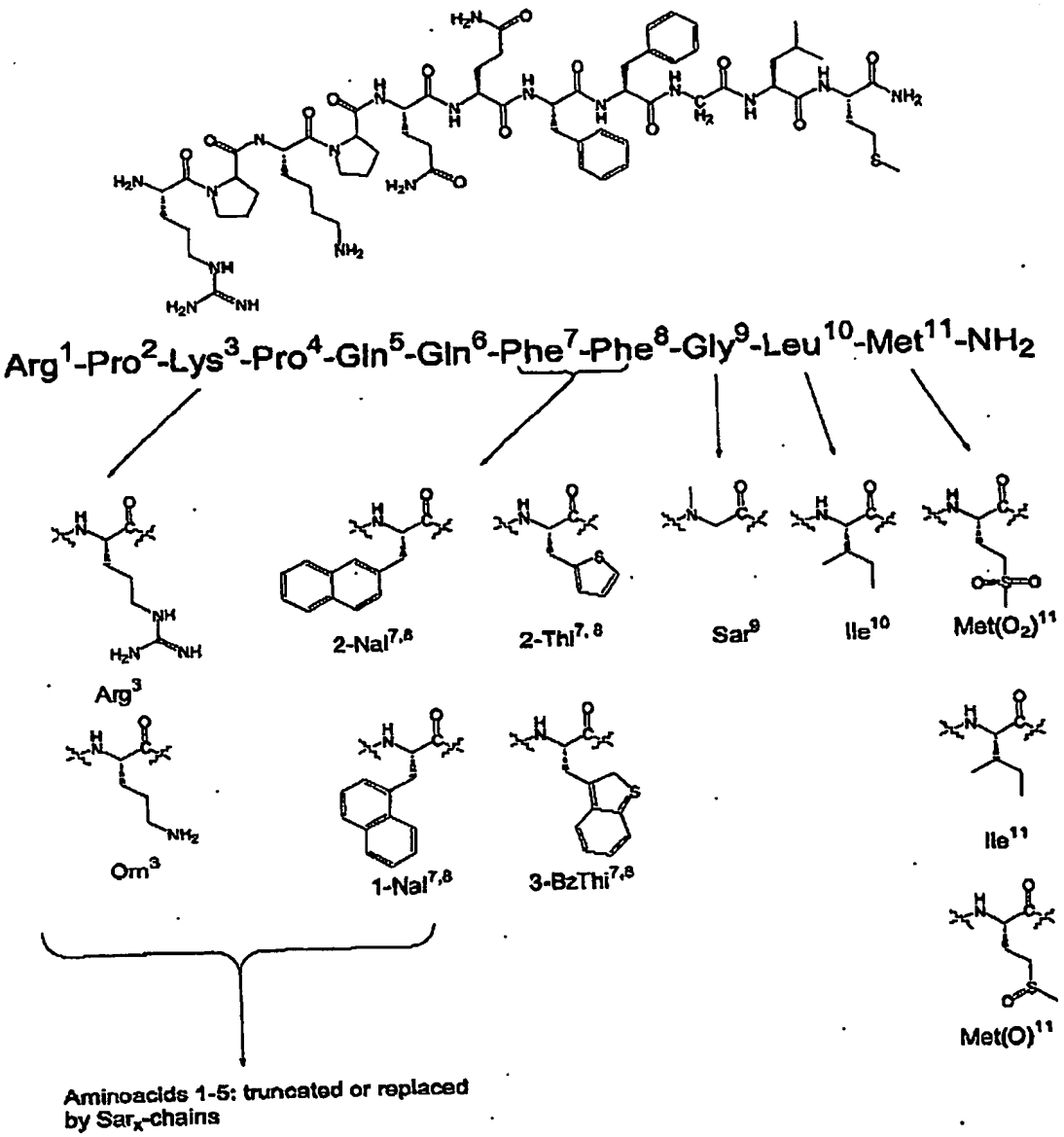
m)) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met(O_2)-NH_2,

n) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met-NH_2,

o) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met(O_2)-NH_2,

with the chelator DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid wherein the substance P and analogue conjugates mentioned under a) –o) are labeled with metal isotopes selected from the group consisting of Actinium-225, Bismut-213, Cupper-64, Gallium-66, Gallium-68, Lutetium-177, Indium-111, Rhenium-186, Rhenium-188, Yttrium-86 and Yttrium-90.

6) new substance P analogues , e.g. undecapeptides embraced by following formula II



II

Legend: 2-Nal: 3-(2-naphthyl)-alanine; 1-Nal: 3-(1-naphthyl)-alanine; 2-Thi: 3-(2-thienyl)-alanine; Sar: sarcosine; Met(O): Methione-sulfoxide; Met(O_2): Methionine-sulfone; 3-BzThi: 3-(3-benzothienyl)-alanine; Orn: ornithine; Ile: isoleucine.

with the exception of the undecapeptide compound of following formula

Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (substance P) and Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met-NH_2 (substance P) or their methionine-sulfoxide (Met(O_2)) derivatives (substance P), being known compounds and as a further embodiment of this invention the process of manufacturing compounds of formula II.

7) New substance P analogues:
a) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met-NH_2,

b) Arg-Pro-Lys-Pro-Gln-Gin-Phe-Thi-Gly-Leu-Met(O_2)-NH_2,

c) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met(O_2)-NH_2,

d) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Sar-Leu-Met(O_2)-NH_2,

e) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met,-NH_2,

f) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met(O_2)-NH_2,

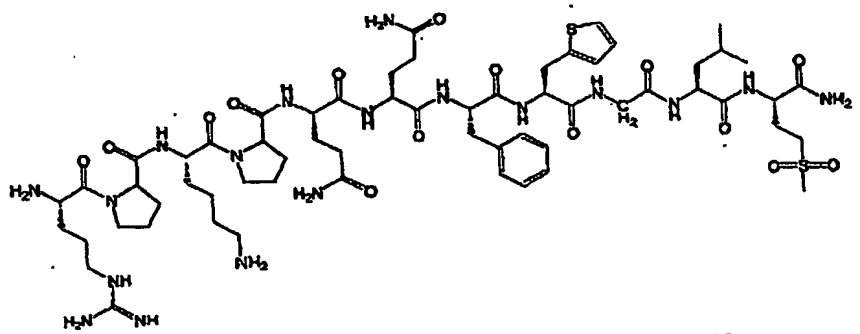
g) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met-NH_2,

h)) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met(O_2)-NH_2,

i) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met-NH_2,

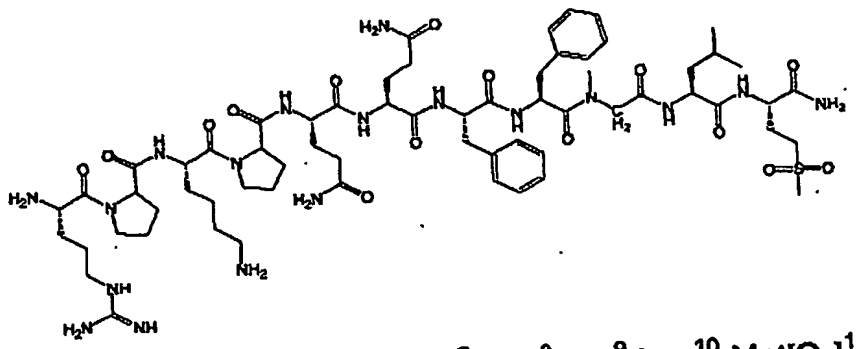
j) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met(O_2)-NH_2,

8) Following peptides were synthesized:
a)



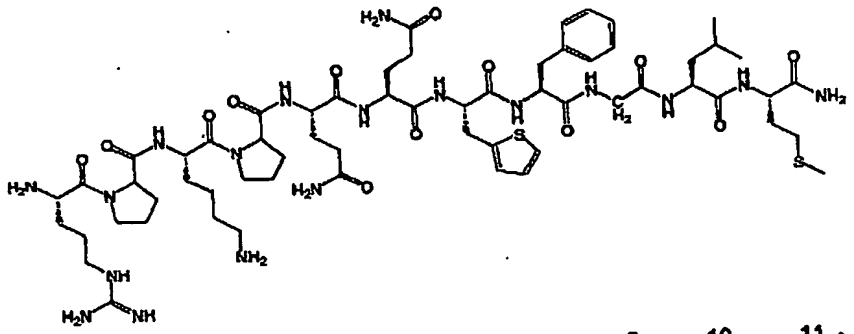
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Phe^{7}-Thi^{8}-Gly^{9}-Leu^{10}-$Met[O_2]^{11}$-NH_2

b)



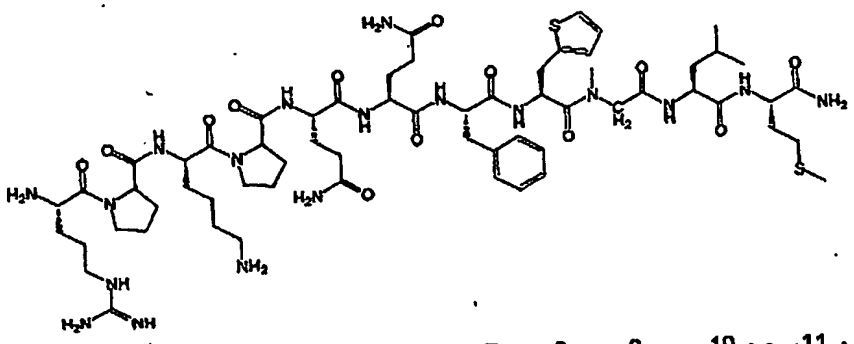
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Phe^{7}-Phe^{8}-Sar^{9}-Leu^{10}-$Met[O_2]^{11}$-NH_2

c)



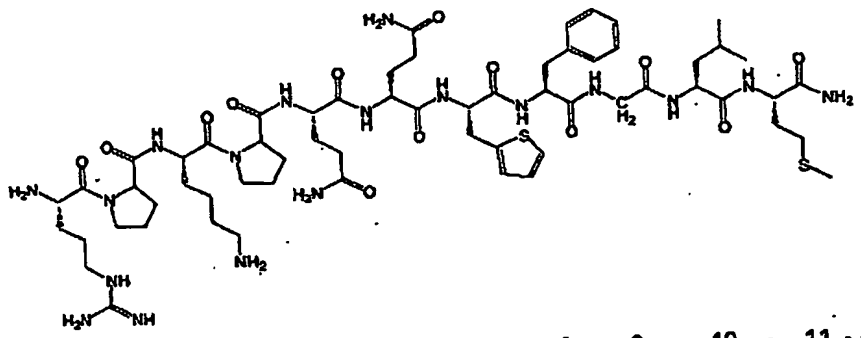
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Thi^{7}-Phe^{8}-Gly^{9}-Leu^{10}-Met^{11}-NH_2

d)



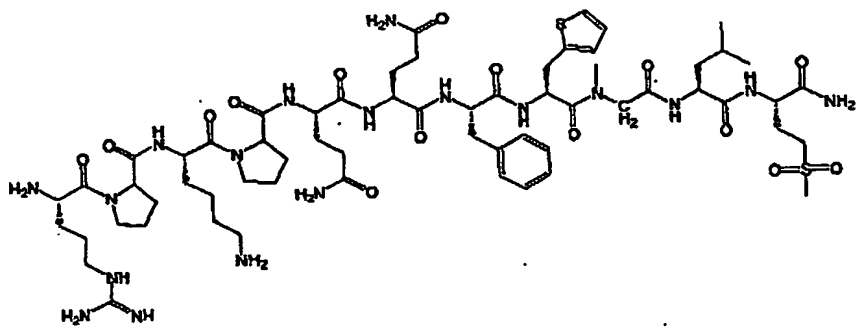
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Phe^{7}-Thi^{8}-Sar^{9}-Leu^{10}-Met^{11}-NH_2

e)



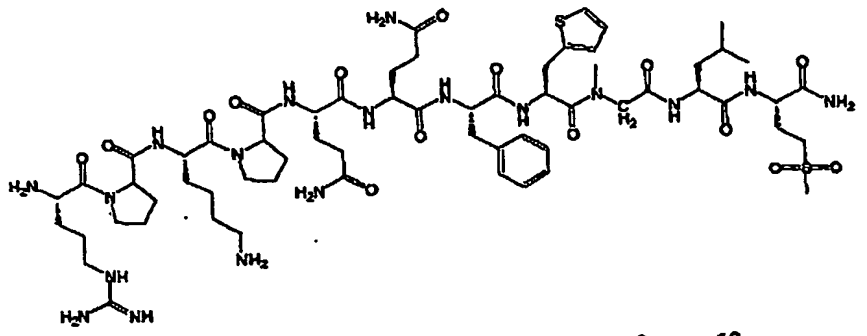
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Thi^{7}-Phe^{8}-Gly^{9}-Leu^{10}-Met^{11}-NH_2

f)



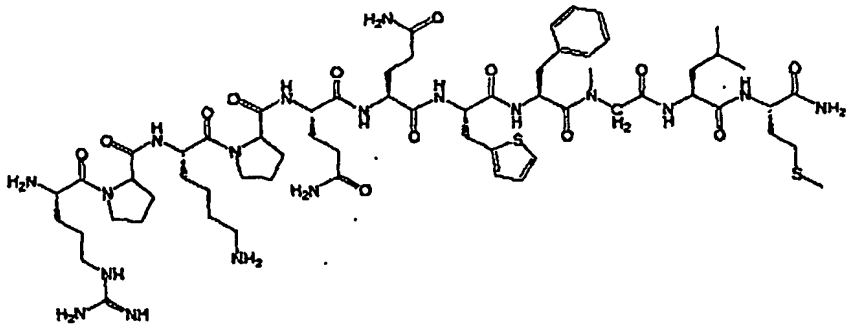
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Phe^{7}-Thi^{8}-Sar^{9}-Leu^{10}-$Met[O_2]^{11}$-NH_2

g)



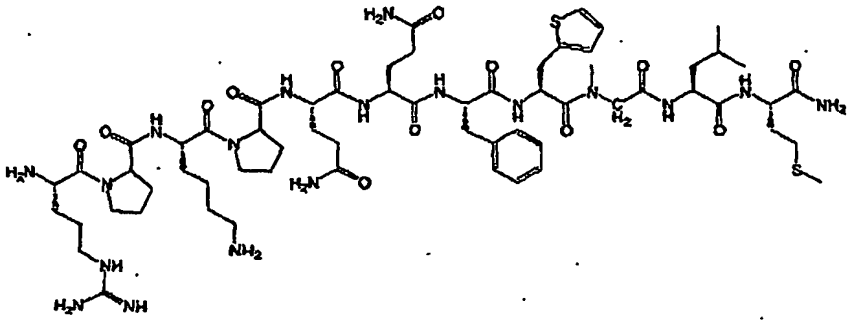
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Phe^{7}-Thi^{8}-Sar^{9}-Leu^{10}-$Met[O_2]^{11}$-NH_2

h)



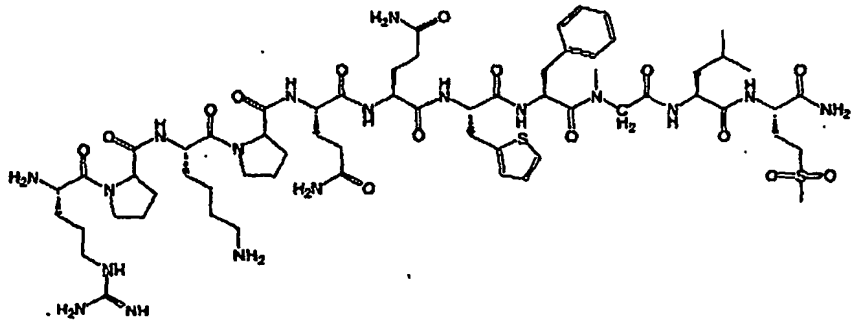
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Thi^{7}-Phe^{8}-Sar^{9}-Leu^{10}-Met^{11}-NH_2

i)



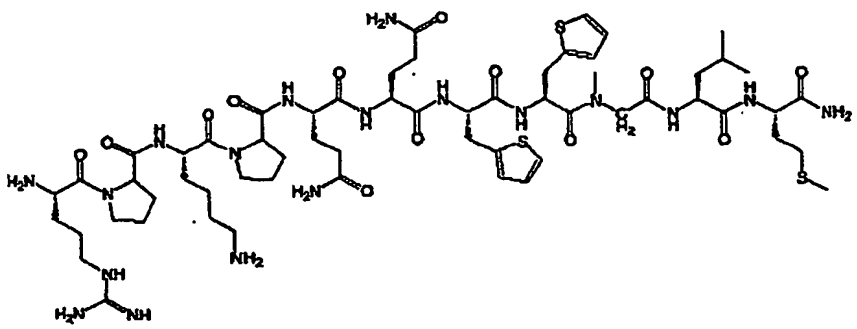
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Phe^{7}-Thi^{8}-Sar^{9}-Leu^{10}-Met^{11}-NH_2

j)



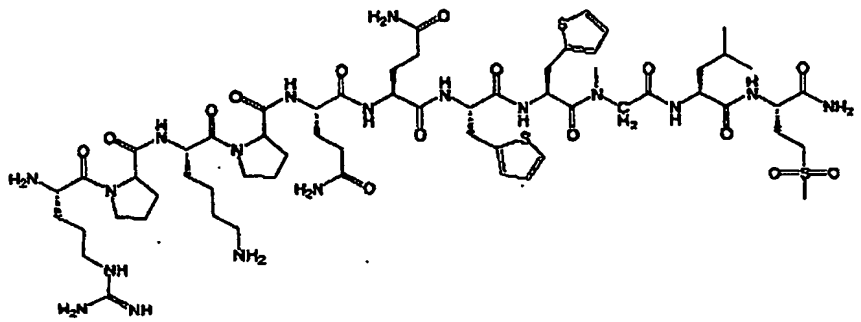
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Thi^{7}-Phe^{8}-Sar^{9}-Leu^{10}-$Met[O_2]^{11}$-NH_2

k)



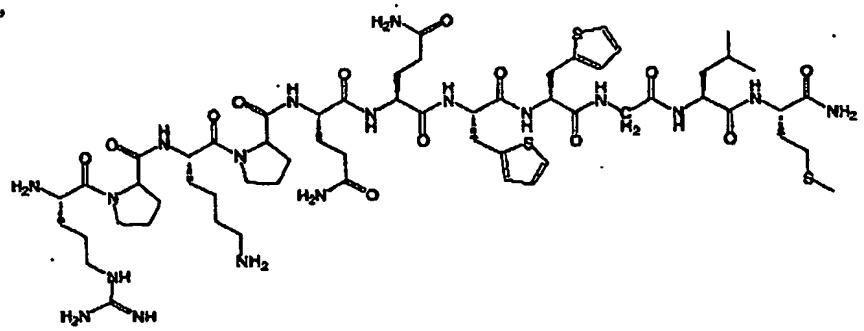
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Thi^{7}-Thi^{8}-Sar^{9}-Leu^{10}-Met^{11}-NH_2

l)



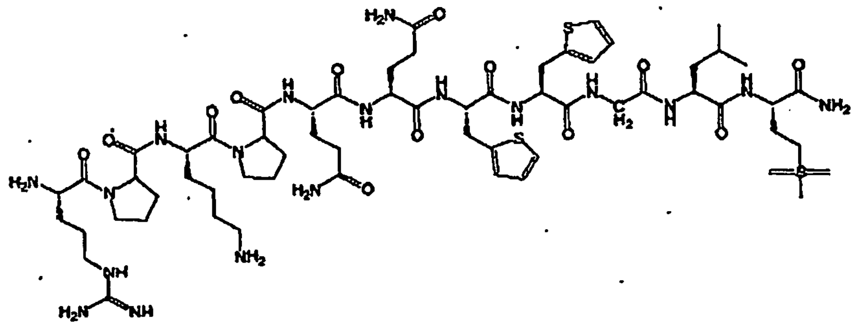
Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Thi^{7}-Thi^{8}-Sar^{9}-Leu^{10}-$Met[O_2]^{11}$-NH_2

m),



Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Thi^{7}-Thi^{8}-Gly^{9}-Leu^{10}-Met^{11}-NH_2

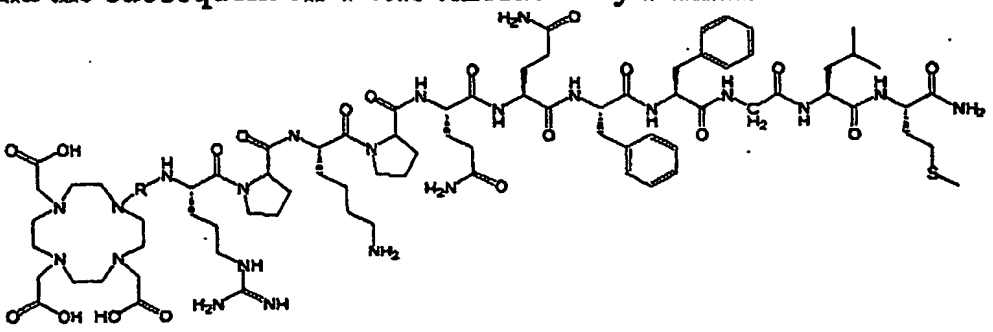
n)



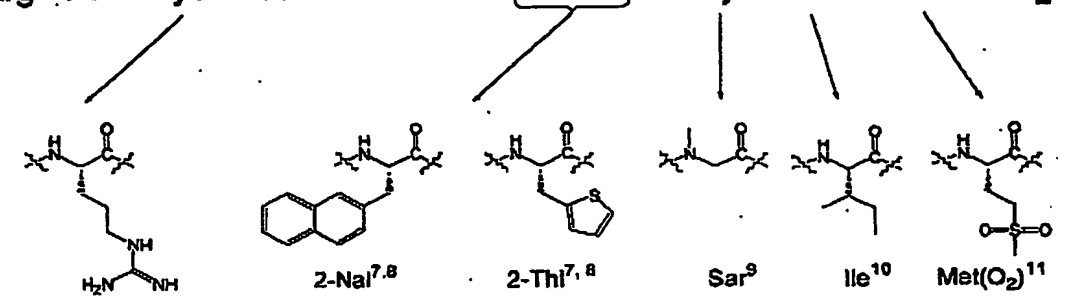
Arg[1]-Pro[2]-Lys[3]-Pro[4]-Gln[5]-Gln[6]-Thi[7]-Thi[8]-Gly[9]-Leu[10]-Met[O$_2$][11]-NH$_2$

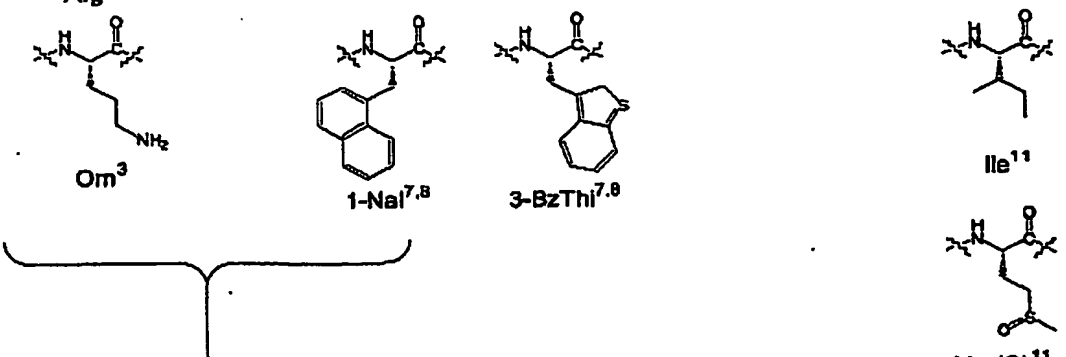
What we claim is :

1) New radiolabeled conjugates based on substance P and analogues thereof with the prochelator DOTAGA(tBu)4 (1-(1-carboxy-3-carboxytertbutoxypropyl)-4,7,10 (carbotertbutoxymethyl)-1,4,7,10-tetraazacyclododecane or prochelator DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid and the subsequent chelators embraced by formula I

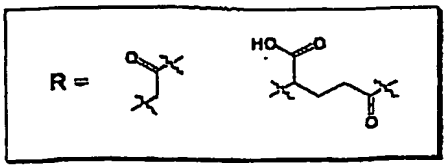


Chelator-Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH_2





Aminoacids 1-5: truncated or replaced by Sar$_X$-chains

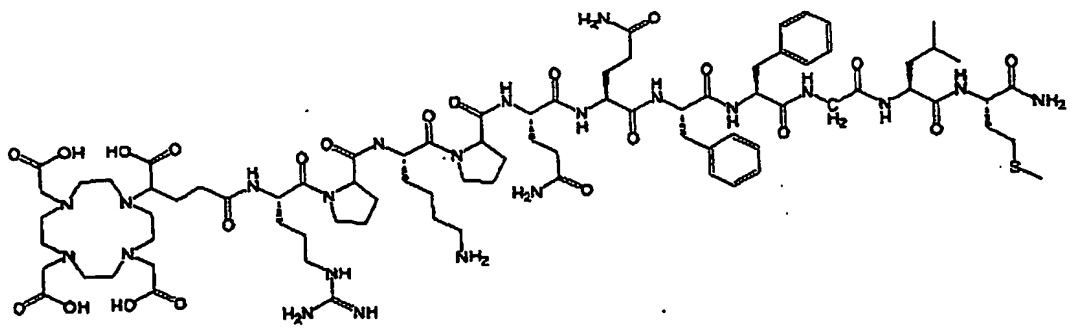


I

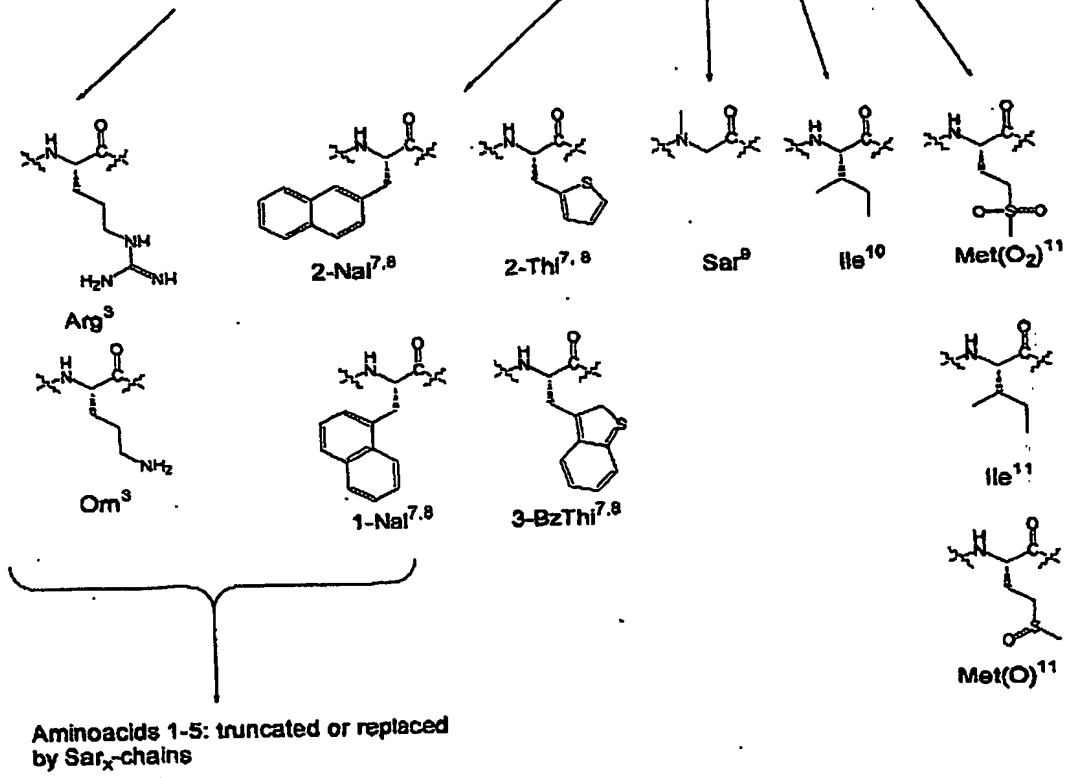
Legend: 2-Nal: 3-(2-naphthyl)-alanine; 1-Nal: 3-(1-naphthyl)-alanine; 2-Thi: 3-(2-thienyl)-alanine; Sar: sarcosine; Met(O): Methionine-sulfoxide; Met(O_2): Methionine-sulfone; 3-BzThi: 3-(3-benzothienyl)-alanine; Orn: ornithine; Ile: isoleucine.

wherein the substance P and anlogue conjugates of formula I are labeled with metal isotopes selected from the group consisting of Actinium-225, Bismut-213,Cupper-64, Gallium-66, Gallium-68, Lutetium-177, Indium-111, Rhenium-186, Rhenium-188, Yttrium-86 and Yttrium-90 or the like known for radiolabeling and the pharmaceutically acceeptable salts and complexes of the radiolabeled conjugates of formula I.

2) New radiolabeled conjugates based on substance P and analogues thereof with the prochelator DOTAGA(tBu)4 (1-(1-carboxy-3-carboxytertbutoxypropyl)-4,7,10 (carbotertbutoxymethyl)-1,4,7,10-tetraazacyclododecane of formula Ia



DOTAGA-Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Phe^{7}-Phe^{8}-Gly^{9}-Leu^{10}-Met^{11}-NH$_2$

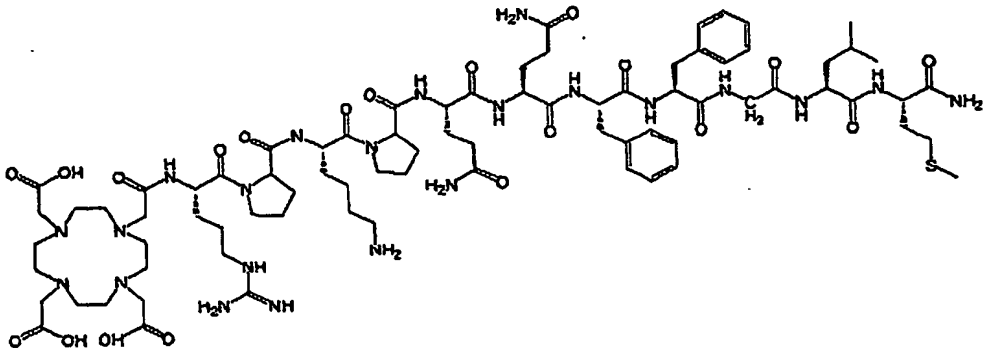


Ia

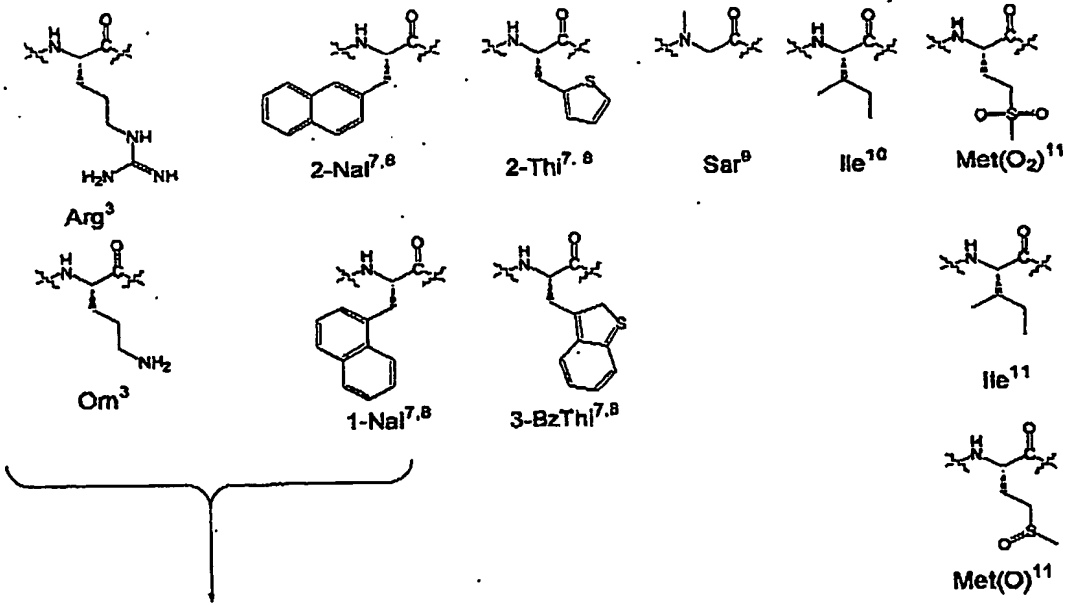
wherein the substance P and analogue conjugates of formula Ia are labeled with metal isotopes selected from the group consisting of Actinium-225, Bismut-213,Cupper-64, Gallium-66, Gallium-68, Lutetium-177, Indium-111, Rhenium-186, Rhenium-188, Yttrium-86 and Yttrium-90 and the

pharmaceutically acceptable salts and complexes of the radiolabeled conjugates of formula Ia.

3) New radiolabeleed conjugates based on substance P and analogues thereof with the prochelator DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid embraced by formula Ib



DOTA-Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH$_2$



wherein the substance P and analogue conjugates of formula Ib are labeled with metal isotopes selected from the group consisting of Actinium-225, Bismut-213,Cupper-64, Gallium-66, Gallium-68, Lutetium-177, Indium-111, Rhenium-186, Rhenium-188, Yttrium-86 and Yttrium-90 and the pharmaceutically acceptable salts and complexes of the radiolabeled conjugates of formula Ib.

4) New radiolabeled conjugates of substance P and analogues selected from the group of undecapeptides:

a) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

b) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met(O_2)-NH_2,

c) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met-NH_2,

d) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Gly-Leu-Met-NH_2,

e) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met-NH_2

f) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met(O_2)-NH_2,

g) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Gly-Leu-Met(O_2)-NH_2,

h) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met(O_2)-NH_2,

i) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Sar-Leu-Met(O_2)-NH_2,

j) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met,-NH_2,

k) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met(O_2)-NH_2

l) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met-NH_2,

m) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met(O_2)-NH_2,

n) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met-NH_2

o) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met(O_2)-NH_2

with the prochelator DOTAGA(tBu)4 (1-(1-carboxy-3-carboxytertbutoxypropyl)-4,7,10 (carbotertbutoxymethyl)-1,4,7,10-tetraazacyclododecane and the subsequent chelator, wherein the substance P and analogue conjugates mentioned under a) –o) are labeled with metal isotopes selected from the group consisting of Actinium-225, Bismut-213,Cupper-64, Gallium-66, Gallium-68, Lutetium-177, Indium-111, Rhenium-186, Rhenium-188, Yttrium-86 and Yttrium-90 and the pharmaceutically acceptable salts and complexes thereof.

5) New radiolabeled conjugates of substance P and analogues selected from the group of undecapeptides:

a) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

b) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met(O_2)-NH_2,

c) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met-NH_2,

d) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Gly-Leu-Met-NH_2,

e) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met-NH_2

f) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met(O_2)-NH_2,

g) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Gly-Leu-Met(O_2)-NH_2,

h) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met(O_2)-NH_2,

i) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Sar-Leu-Met(O_2)-NH_2,

j) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met,-NH_2,

k) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met(O_2)-NH_2

l) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met-NH_2,

m) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met(O_2)-NH_2,

n) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met-NH_2

o) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met(O_2)-NH_2

with the prochelator DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid and the subsequent chelator, wherein the substance P and analogue conjugates mentioned under a) –o) are labeled with metal isotopes selected from the group consisting of Actinium-225, Bismut-213,Cupper-64, Gallium-66, Gallium-68, Lutetium-177, Indium-111, Rhenium-186, Rhenium-188, Yttrium-86 and Yttrium-90 and the pharmaceutically acceptable salts and complexes thereof.

6) A new radiolabeled conjugate of Ar-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met-NH_2 (substance P) with the prochelator (chelator) DOTAGA(tBu)4 (1-(1-carboxy-3-carboxytertbutoxypropyl)-4,7,10 (carbotertbutoxymethyl)-1,4,7,10-tetraazacyclododecane and the pharmaceutically acceptable salts and complexes thereof.

7) A new radiolabeled conjugate of Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met(O_2)-NH_2, (substance P) with the prochelator (chelator) DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid
and the pharmaceutically acceptable salts and complexes thereof.

8) A new radiolabeled conjugate of Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met-NH_2,(substance P) with prochelator (chelator) DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid and the pharmaceutically acceptable salts and complexes thereof.

9) A new radiolabeled conjugate of Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Gly-Leu-Met-NH_2, (substance P) with prochelator (chelator) DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid and the pharmaceutically acceptable salts and complexes thereof.

10) A new radiiolabeled conjugate Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met-NH_2 (substance P) with prochelator (chelator) DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid and the pharmaceutically acceptable salts and complexes thereof.

11) A new radiolabeled conjugate Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met(O_2)-NH_2 (substance P) with prochelator (chelator) DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid and the pharmaceutically acceptable salts and complexes thereof.

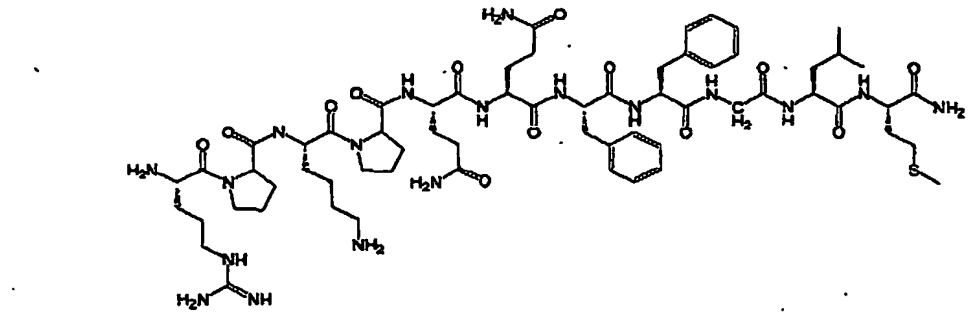
12) A new radiolabeled conjugate Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Gly-Leu-Met(O_2)-NH_2 (substance P) with prochelator (chelator) DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid and the pharmaceutically acceptable salts and complexes thereof.

13) A new radiolabeld conjugate Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Sar-Leu-Met-NH_2 (substance P) with prochelator (chelator) DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid and the pharmaceutically acceptable salts and complexes thereof.

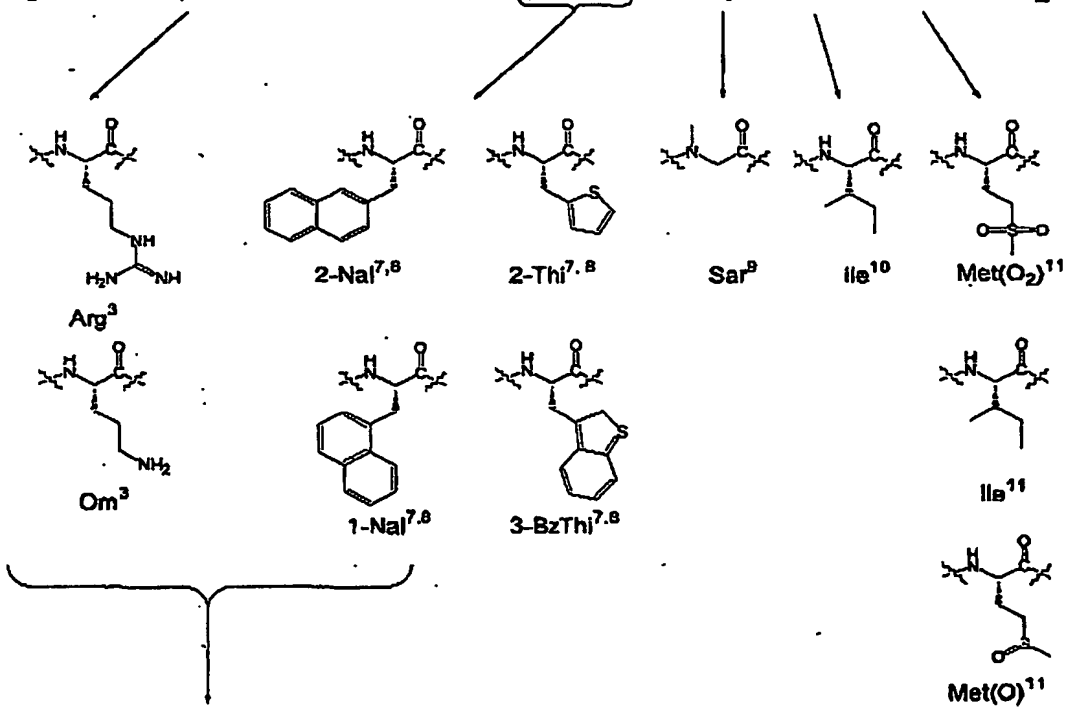
14) A new radiolabeled conjugate Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met-NH_2 (substance P) with prochelator (chelator) DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid and the pharmaceutically acceptable salts and complexes thereof.

15) Process for the preparation of new radiolabeled conjugates based on substance P and analogues with the prochelator DOTAGA(tBu)4 (1-(1-carboxy-3-carboxytertbutoxypropyl)-4,7,10 (carbotertbutoxymethyl)-1,4,7,10-tetraazacyclododecane or the prochelator DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid and the subsequent chelators of formula I defined under claim 1, characterised by preparing by solid phase peptide synthesis (SPPS) the undecapeptide carried out for example by using Fmoc (9-fluorenemethoxycarbonyl) strategy, coupling with the prochelator and labeling with a metal iostope.

16) New substance P analogues being undecapeptides of formula II



Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH_2



II

Legend: 2-Nal: 3-(2-naphthyl)-alanine; 1-Nal: 3-(1-naphthyl)-alanine; 2-Thi: 3-(2-thienyl)-alanine; Sar: sarcosine; Met(O): Methione-sulfoxide;

Met(O_2): Methionine-sulfone; 3-BzThi: 3-(3-benzothienyl)-alanine; Orn: ornithine; Ile: isoleucine.

with the exception of the undecapeptide compound of following formula Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (substance P) and Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met-NH_2 ([Sar^9]- substance P) or their methionine-sulfoxide (Met(O_2)) derivatives, being known compounds.

17) New substance P analogues selected from the group of undecapeptides:

a) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met-NH_2,

b) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Gly-Leu-Met(O_2)-NH_2,

c) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Gly-Leu-Met(O_2)-NH_2,

d) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Thi-Sar-Leu-Met(O_2)-NH_2,

e) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met,-NH_2,

f) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Phe-Sar-Leu-Met(O_2)-NH_2,

g) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met-NH_2,

h)) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Sar-Leu-Met(O_2)-NH_2,

i) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met-NH_2,

j) Arg-Pro-Lys-Pro-Gln-Gln-Thi-Thi-Gly-Leu-Met(O_2)-NH_2,

and the pharmaceutically acceptable salts and complexes thereof.

18) Process for the preparation of substance P analogues of formula II, characterised by preparing by solid phase peptide synthesis (SPPS) the undecapeptide, carried out for example by using Fmoc (9-fluorenemethoxycarbonyl) strategy.

19) Use of new radiolabeled conjugates of formula I for targeting and treatment of brain tumors.

20) Use of new radiolabeled conjugates of formula I for targeting and treatment of gliomas.

21) Use of new radiolabeled conjugates of formula Ia for targeting and treatment of brain tumors.

22) Use of new radiolabeled conjugates of formula Ia for targeting and treatment of gliomas.

23) Use of new radiolabeled conjugates of formula Ib for targeting and treatment of brain tumors.

24) Use of new radiolabeled conjugates of formula Ib for targeting and treatment of gliomas.

25) Use of new radiolabeled conjugates as claimed in claim 4 for targeting and treatment of brain tumors.

26) Use of new radiolabeled conjugates as claimed in claim 4 for targeting and treatment of gliomas.

27) Use of new radiolabeled conjugates as claimed in claim 5 for targeting and treatment of brain tumors.

28) Use of new radiolabeled conjugates as claimed in claim 5 for targeting and treatment of gliomas.

29) Use of a substance P analogue as claimed in claim 17 for targeting and treatment of brain tumors.

30) Use of a substance P analogue as claimed in claim 17 for targeting and treatment of gliomas.

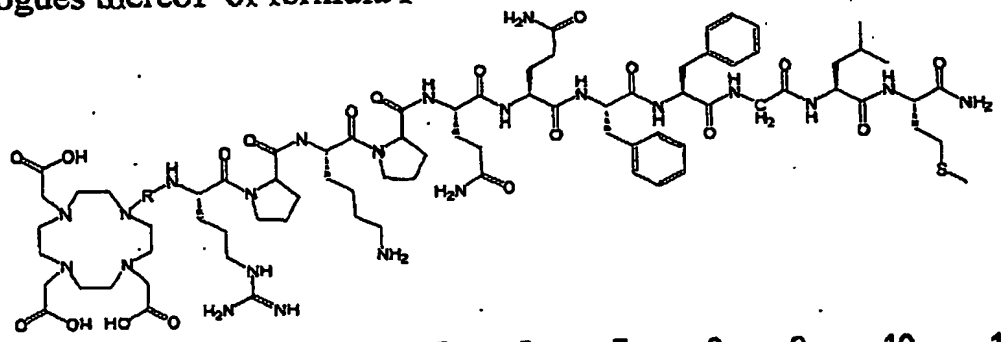
31) Use of new substance P analogues of formula II as claimed under claim 16 for the preparation of new radiolabeled conjugates of formula I, Ia or Ib.

32) Use of the new conjugates of formula I, Ia, and Ib and of the new substance P analogues of formula II for preparing medicaments for targeting and treatment brain tumors.

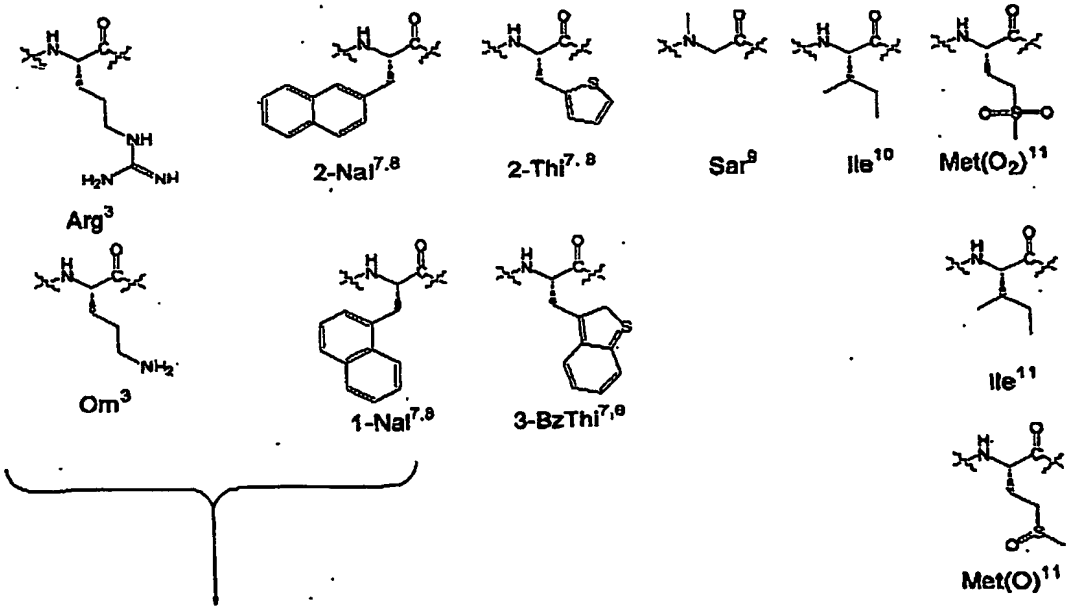
33) Use of the new conjugates of formula I, Ia, and Ib and of the new substance P analogues of formula II for preparing medicaments for targeting and treatment of gliomas.

Abstract

The invention relates to new radiolabeled conjugates based on substance P and analogues thereof of formula I

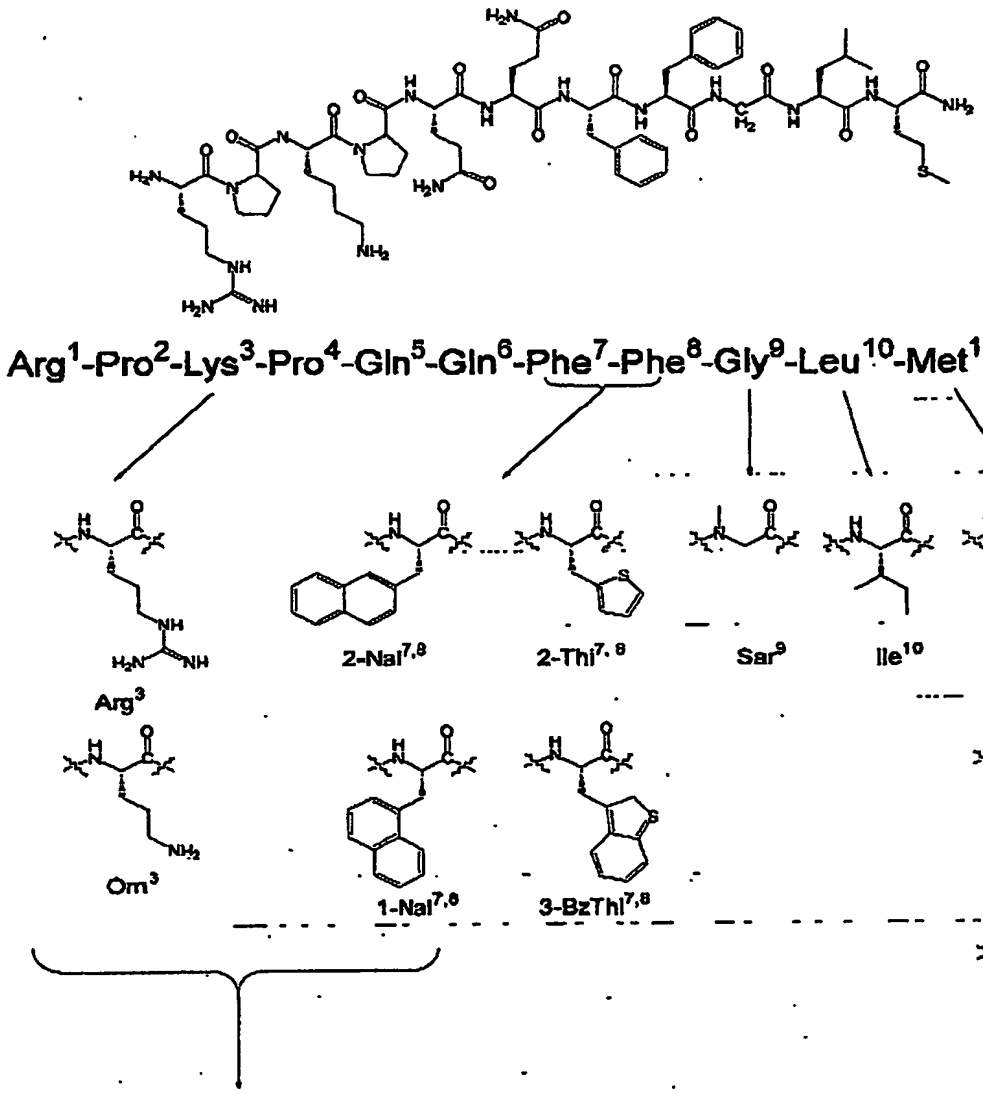


Chelator-Arg^{1}-Pro^{2}-Lys^{3}-Pro^{4}-Gln^{5}-Gln^{6}-Phe^{7}-Phe^{8}-Gly^{9}-Leu^{10}-Met^{11}-NH$_2$



with the prochelator DOTAGA(tBu)4 (1-(1-carboxy-3-carboxytertbutoxypropyl)-4,7,10 (carbotertbutoxymethyl)-1,4,7,10-tetraazacyclododecane or the prochelator DOTA (^{t}Bu)$_3$(1,4,7,10-tetraazaacyclo-dodecane-1,4,7,-tris(acetic acid-t-butyl ester)-10-acetic acid and the subsequent chelators and the use thereof as medicament for targeting and treatment of brain tumors, e.g.gliomas.

Furthermore the invention refers to substance P anlogues being undecapeptides of formula II with the exception of the undecapeptide compound of following formula) Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (substance P) and Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Sar-Leu-Met-NH_2 ([Sar^9]- substance P) or their methionine-sulfoxide (Met(O_2)) derivatives



and the use thereof as medicament for targeting and treatment of brain tumors, e.g. gliomas.